

2015

Journal of the Arkansas Academy of Science - Volume 69 2015

Academy Editors

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Journal of the
**ARKANSAS ACADEMY
OF SCIENCE**

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ARKANSAS ACADEMY OF SCIENCE
ARKANSAS TECH UNIVERSITY
DEPARTMENT OF PHYSICAL SCIENCES
1701 N. BOULDER AVE
RUSSELLVILLE, AR 72801-2222

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Arkansas Academy of Science, Dept. of Physical Sciences, Arkansas Tech University
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C. E. Hoffman	1959	Gary Heidt	1986	Marc Seigar	2013
N. D. Buffaloe	1960	Edmond Bacon	1987	Jeff Robertson	2014
H. L. Bogan	1961	Gary Tucker	1988	Abdel Bachri	2015

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ARKANSAS STATE UNIVERSITY, Jonesboro	UNIVERSITY OF ARKANSAS AT MONTICELLO
ARKANSAS TECH UNIVERSITY, Russellville	UNIVERSITY OF ARKANSAS AT PINE BLUFF
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UNIVERSITY OF ARKANSAS AT FORT SMITH	Little Rock
UNIVERSITY OF ARKANSAS AT FAYETTEVILLE	

EDITORIAL STAFF

<i>Editor-in-Chief</i>	<i>Managing Editor</i>	<i>Biota Editor</i>	<i>Associate Editors</i>
Mostafa Hemmati P.O. Box 1950 Russellville, AR 72811 mhemmati@atu.edu	Ivan H. Still Dept. of Biological Sciences Arkansas Tech University Russellville, AR 72801 istill@atu.edu	Douglas A. James Dept. of Biological Sciences Univ. of Arkansas Fayetteville, AR 72701 djames@uark.edu	C. Geren, UAF F. Hardcastle, ATU

COVER: Red Crossbills eating algae (Cladophora sp.) at the Fayetteville Country Club on 10 December 2012 from Red Crossbill Invasion of Northwestern Arkansas During 2012-2013 by K.G. Smith, J.C. Neal, and M.A. Young pp 83-87

ARKANSAS ACADEMY OF SCIENCE 2015



APRIL 10-11, 2015
99th ANNUAL MEETING

Henderson State University
Arkadelphia, Arkansas

JOURNAL ARKANSAS ACADEMY OF SCIENCE

Annual Meeting April 10-11, 2015
Henderson State University

Abdel Bachri
President

Ann Willyard
President-Elect

Edmond Wilson
Vice-President

Jeff Robertson
Secretary

Mostafa Hemmati
Treasurer

Mostafa Hemmati
JAAS
Editor-in-Chief

Ivan Still
JAAS
Managing Editor

R. Panneer Selvam
Newsletter Editor

Salomon Itza
Webmaster

Collis Geren
Historian

Secretary's Report MINUTES OF THE 99th MEETING

ARKANSAS ACADEMY OF SCIENCE
SPRING 2015 BUSINESS MEETING MINUTES
April 11, 2015 – 11:30 am
Henderson State University

1. The meeting was called to order by President Abdel Bachri.

2. Local Arrangements Committee: Martin Campbell
95 people pre-registered for the meeting representing 21 universities. This meeting is held concurrently with the Arkansas Undergraduate Research Conference and has 60 registrants representing 7 universities giving 16 oral presentations and 20 posters. For the AAS meeting there were 45 oral presentations and 36 poster presentations. Total in attendance of about 180.

Discussion

It would be useful if abstract submission, payment of AAS dues, etc. could be done online through the website and be a more automated process. Panneer investigated some of these issues and reported that the paypal service said if it is a non-profitable organization then the charge for each payment is 2.2%+\$0.3. If it is not non-profit then it is 2.9%+\$0.3. If the membership payment can be increased this could be a method of payment collection without much trouble. Also the registration also can be done if one is interested.

3. Secretary's Report: Jeff Robertson

Minutes from the 2014 Fall Executive Committee Meeting in December were reviewed and accepted.

Prior to registration at this meeting, the Academy had 107 members (49 of which are life members).

4. Treasurer's Report: Mostafa Hemmati

An accounting of the AAS for 2014 was presented and discussed by the membership. The report was reviewed by an auditing team made of selected members of the Academy and accepted by the membership (see AAS financial statement in appendix.)

5. Historian's Report: Collis Geren

The 2015 spring meeting of the Arkansas Academy of Science at Henderson State University in Arkadelphia, Arkansas is the 99th annual meeting of the Academy. This will mark the sixth time that Henderson will have hosted the Academy having done so previously in 2008, 1993, and 1982 and in 1941 and 1935 when named Henderson State Teachers College.

Henderson State University is the second oldest public four year degree granting institution in Arkansas. It was founded in 1890 as Arkansas Methodist College. In 1904 the institution was renamed for Charles Christopher Henderson, a prominent Arkadelphia businessman. In 1911 the institution was renamed as Henderson-Brown College to also honor Walter Brown. Some 39 years later the institution was closed and combined with Hendrix College in Conway. Arkadelphians and other Arkansans disagreed with this plan and Henderson State Teachers College was created in 1929 as a public institution. To reflect Henderson's developing mission the name was changed to Henderson State College in 1967 and to Henderson State University in 1976.

Henderson has had 17 presidents with Glen Jones the most recent to hold that position.

Henderson State University had an enrollment of 3,627 for the fall of 2014. The institution offers 42

Business meeting report

bachelor's degrees, 11 master's, 1 educational specialist degree, and 5 certificate programs.

The mission of Henderson State University is to provide a learning environment that prepares students for a lifetime of intellectual and personal growth in an increasingly global society. This bridges students' academic aspirations to career success by integrating professional studies and the liberal arts.

The Academy is indebted to Professor Martin Campbell for leading Henderson in hosting the 99th annual meeting of the Academy.

6. Journal (JAAS #68) Report: Editor-In-Chief Mostafa Hemmati

During the spring 2014 semester, 36 manuscripts were submitted for consideration for publication in volume 68 of the Journal of the Arkansas Academy of Science (JAAS). Soon after receiving the manuscripts, all manuscripts were sent to reviewers and two Associate Editors. The reviewers sent all manuscripts and their comments back before the end of July 2014.

Reviewers' comments were sent to the authors between July 15, 2014, and July 30, 2014. That process was completed by July 30, 2014. The authors were asked to respond to the reviewers' comments and return their manuscript back to Managing Editor, Dr. Still, by August 31, 2014. That allowed more than a month of time for the authors to respond to the reviewers' comments. In the same letter, the authors were also asked to mail a check for their page charges. August 31, 2014, was also the deadline for receipt of the payment of the page charges.

Six manuscripts required major revisions, and at the end, five were rejected. The remaining manuscripts needed minor revisions. For one of the manuscripts, we did not receive the page charges in a reasonable length of time; therefore, that manuscript can be considered for volume 69 of the Journal. Also, one article which we had not received the page charges on time to be included in volume 67 of the Journal, was included in volume 68. Therefore, volume 68 of the Journal includes 31 manuscripts. In the process of manuscript submission, no manuscripts were lost.

Two Associate Editors, Dr. Collis Geren and Dr. Frank Hardcastle, helped considerably with locating possible reviewers for the manuscripts or serving as reviewer for more than one manuscript. I am grateful for both Associate Editors' assistance. All

activities relating to the handling of the manuscripts were performed electronically, and on the whole this expedited the review process. Managing editor post was performed by Dr. Ivan Still and as usual he did an excellent job. The Journal was completed by December 30, 2014. Printing of the Journal was completed by March 20, 2015. I have used the Russellville Printing Company for printing of the Journal in the past and I used them again for printing volume 68 of the Journal.

Managing Editor Ivan Still

The Managing Editor's report was submitted and summarized in the report given by the Editor-In-Chief. One additional observation, based on communications with four of the corresponding authors whose papers were rejected: I suspect that these papers will not be resubmitted as the authors seem to be leaving/left their position after the manuscripts were returned in July. This situation may also account for the author of one paper that was accepted this year did not submit the page charges.

As of the start of the meeting, 25 manuscripts had been submitted for consideration of publication in volume 69 (2016) of the JAAS. It should be noted that at least one of the authors needs to be an AAS member to publish in the JAAS as well as a presentation being made at an Academy meeting.

7. Webmaster: Salomon Itza

The webmaster has been updating the webpage to provide information on the 99th annual meeting of the Academy. The information was provided by Dr. Martin Campbell from HSU. The webmaster also has updated the Newsletter link to include the latest version developed by Dr. Panneer Selvam.

The account purchased with the company IPAGE.COM, the current host, will expire on June 06 2015. The president asked that research be done on the possibility to consolidate the host service and the domain server. The domain server is hosted by the company REGISTER.COM. This server hosts the public portal: www.arkansasacademyofscience.org. They charge \$119.50 for hosting the website and for domain registration. To keep REGISTER.COM as the host will cost about \$40.00 more per year; compared to the costs of IPAGE.COM. This is a small price when considering that all files will be located in one single server. The webmaster also keeps backups of files on his personal computer, which he will

Business meeting report

surrender if someone else is appointed as webmaster.

Finally, the suggestion to have the AAS website as the host for online registration has been made before. To avoid that the webmaster is caught in the middle of local arrangements, registration and other details; the preference would be that local host takes full responsibility of the registration.

8. Newsletter: Panneer Selvam

Panneer explored assignment of university liaisons to help promote communication of Academy activities throughout the state institutions as well as some suggestions to identify those persons at all 4-year institutions.

Duties as Liaison for the AAS & Requirements.

1. Expected to attend the AAS meeting. If two consecutive years no attendance and others are actively participating then that person will replace the position.
2. Every five years we look for alternate if necessary.
3. Circulate the news about AAS meeting and other news to the campus.
4. Be the contact point for promoting AAS and get new members.
5. Also solicit papers for the journal.
6. Help get their school to join AAS as an Institutional Member

List of Universities to be Considered & the Liaison Officer Name

1. Arkansas Tech (Dr. Scott W. Kirkconnell, Professor of Biology, 1701 North Boulder Ave., Russellville, AR 72801-2222, email: skirkconnell@atu.edu ph:479-968-0675)
2. Ouachita Baptist University (Dr. Jess Kelly, Assistant Professor of Biology, 410 Ouchita Street, Box 3792, Arkadelphia, AR 71998-0001, email: kellyj@obu.edu , ph: 870-245-4187)
3. Philander Smith College (Dr. Frank Hahn, Associate Professor of Chemistry, 900 Daisy Bates Drive, Little Rock, AR 72202, email: fhahn@philander.edu , ph: 501-975-8511)
4. Southern Arkansas University (Dr. Abdel Bachri, Associate Professor, Department of Engineering Physics, P.O. Box 9256, Magnolia, AR 71754-9256, email: agbachri@saumag.edu , ph: 870-235-4283, cell: 870-949-5726)

5. University of Arkansas (Dr. R. Panneer Selvam, University Professor & Womble Professor of Computational Mechanics and Nano tech., Department of Civil Engineering, BELL 4190 University of Arkansas, Fayetteville, AR 72701, email: rps@uark.edu , ph: 479-575-5356)
6. University of the Ozarks (Dr. Salomon Itza, Assistant Professor of Physics, 415 N. College Avenue, Clarkson, AR 72830, email: sitza@ozarks.edu , ph: 479-979-1365)
7. Arkansas State University - Dr. Hashim Ali
8. UCA – Stephen Addison
9. JBU
10. UALR
11. UAFS
12. Harding – Ed Wilson
13. Hendrix – Matt Moran
14. Lyon
15. UAMS
16. Henderson State University– Marty Campbell

9. Committee Reports:

Nominations Committee: Mostafa Hemmati

Ann Willyard inherited the presidency of the Academy, with Ed Wilson as President-elect, Abdel Bachri as Past-President, and Panneer Selvam as Vice President.

10. Business Old and New:

In 2016, the 100th annual AAS meeting will be held at the University of Arkansas on April 1-2, 2016 in Fayetteville by host Jim Rankin (VP Research). The AAS is hoping to make this annual meeting a special event with perhaps a nobel laureate as keynote speaker and a welcome by governor or senator.

A host for the future 102nd meeting in 2018 is solicited to the community at large at this time as Stephen Addison (UCA) has tentatively agreed to look into hosting the 101st meeting in the new science building on the campus in Conway.

AAS Undergraduate Research Awards (\$500) were given to support undergraduate research. Recipients of the 2015 awards were Kaitlin Gaiser (HSU), Mallory Bell (HSU), Karemera Hassan (Hendrix) and Julia Lefler (Hendrix).

The membership began the process of changing the AAS By-Laws (#7) to allow membership to be identified as in other professional organizations (Jan.-Dec. calendar year) rather than April to April.

Business meeting report

Please be more active by helping solicit faculty (especially younger faculty) to consider becoming session chairs at the Academy meeting so that they can create a session and help recruit.

Student Awards presented at the 99th AAS meeting were as follows:

Undergrad. Chemistry & Biochemistry: Oral Presentations: Connor Harris (ATU), Jake Windley (Harding), Gunner Klemmer (Harding)

Undergrad. Physical Sciences & Engineering Oral Presentations: Ryan Horn (ATU), Emily Valerio (Harding), Kyun Yoon (Harding)

Grad/Undergrad Life Sciences Oral Presentation: Mary Killmer (ASU), Jillian del Sol (Hendrix), Jessie Kitchens (OBU)

Grad. Physical Sciences & Engineering Oral Presentations: Nefal Ahmed (UA), Michael Newell (ASU), Hussain Seyed (UALR)

Poster Presentation Recognition: Seth St. John (UAM), Lauren Clai (SAU), Casey O'Hara (SAU)

11. Motions and Action Items:

AAS constitution and by-laws revisions were reviewed at Executive Committee Meeting, November 2012 and read for the first time to membership at the spring 2013 meeting. The second reading and vote for adoption occurred at this business meeting of the AAS membership and revisions were approved. The revised AAS constitution and by-laws are available on the Academy website.

Continuation of AAS Undergraduate Research Awards approved.

Considering our electronic publication of the journal and its timely appearance online through <http://libinfo.uark.edu/aas/>, add a checkbox "Do you desire a hardcopy of the JAAS" on the membership form to perhaps reduce journal publication costs and the number of hardcopies generated.

Nominations for AAS Vice-President were received and Panneer Selvam was elected. He will serve a four year rotation through the AAS Executive committee as VP, President-Elect, President and finally Past-President.

The 100th meeting will be your last chance to collect any vintage copies of the JAAS you desire, after the meeting, they will be headed to the recycle bin.

Academy budget 2015-2016 (outside costs associated with Journal publication) approved, up to \$7,000 which includes but not limited to:

- a. AAS Undergraduate Research Grants (up to 5, up to \$500)
- b. AAS Annual spring meeting student presentation awards
- c. AAS Secretary, journal mailings (if requested)
- d. AAAS representative travel (if requested)
- e. Affiliate student awards Junior Academy, AJSHS, Arkansas Science Fair

Ann Willyard inherited the Presidency of the Academy, with Ed Wilson as President-elect, Abdel Bachri as Past-President, and Panneer Selvam as Vice President.

Meeting Adjourned

Jeff Robertson, AAS Secretary

Treasurer's Report ARKANSAS ACADEMY OF SCIENCE 2015 FINANCIAL STATEMENT December 1, 2015

Balance – December 1, 2015	\$110,045.33
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Balance – November 10, 2014	<u>\$103,764.83</u>
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Net Gain	\$6,280.50
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DISTRIBUTION OF FUNDS

Checking Account - Arvest Bank, Russellville	\$8,045.33
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Certificate of Deposit Includes Phoebe and George Harp Endowment Arvest Bank, Russellville	\$51,000.00
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Certificate of Deposit Arvest Bank, Russellville	\$51,000.00
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Combined interest from Arvest Bank alone since 12-01-2015:
 $\$42.55 + \$42.55 = \$85.10$; combined interest from Bank of the
 Ozarks on 12-21-2015, $\$46.46 + \$12.69 + \$52.43 + \$42.87 = \$154.45$

TOTAL	<u>\$110,045.33</u>
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INCOME:

1. Transfer from CD to Checking	\$27,289.47
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2. GIFTS RECEIVED

a. Ouachita National Forest - Sponsorship	-0-
b. Contribution, Collis Geren	\$250

\$250

Business meeting report**3. INTEREST** (Interest Earned Year to Date, ~ December 21, 2015)

a. Checking Account, Bank of the Ozarks, ...	448	\$0
b. CD1 (Bank of the Ozarks),	929	\$46.46
c. CD2 (Bank of the Ozarks), ...	594	\$12.69
d. CD3 (Bank of the Ozarks),	583	\$52.43
e. CD4 (Bank of the Ozarks).....	396	\$42.87
f. CD1 (Arvest Bank)	357	\$42.55
g. CD2 (Arvest Bank)	358	\$42.55

All interest was added to the CDs **\$239.55**

4. JOURNAL

a. Page Charges	\$6,899.42
b. 1 Copy of Vol. 67	\$50
c. Subscriptions, University of Arkansas	\$1,050
d. Journal Subscription Cox Subscription	\$100
e. Journal Contribution – David Saugy	\$100

\$8,199.42

5. MISCELLANEOUS INCOME \$0

6. MEMBERSHIP

a. Associate	\$180
b. Individuals	\$1350
c. Institutional (UAMS, \$100) + Harding (Two Years \$200)	\$300
d. Life (Ben Rowley, \$125, 4 th ; Jacobs \$125, 4 th ; Dan Bullock, \$125, 1 st ; Kelly, Ouschita, \$125 1 st)	\$500
e. Sponsoring	\$45
f. Sustaining	\$105

\$2,480**7. MEETING INCOME**

a. Total Registration and Fees	\$1,095
b. Additional Meeting Income	\$450

\$1,545

TOTAL INCOME \$12,474.42

EXPENSES**1. STUDENT AWARDS**

1. Nawfal Ahmad	\$100
2. Michael Newell	\$50
3. Hussein Sayad	\$50
4. Ryan Horn	\$100
5. Emily Valerio	\$50
6. Kyung Yoon	\$50
7. Conner Harris	\$100
8. Jake Windley	\$50
9. Gunnar A. Klemmer	\$50
10. Mary Kilmer	\$100
11. Jillian F del Sol	\$50
12. Jessie Kitchens	\$50

\$800**2. AWARDS (Organizations)**

a. Junior Science and Humanities Sym.	\$400
b. Arkansas State Science Fair	\$400
c. Arkansas Junior Academy of Science	\$250
d. Arkansas Science Talent Search	\$150

\$1,200**3. UNDERGRADUATE RESEARCH AWARDS**

a. Dr. Willyard - Hassa, Hendrix	\$500
b. Dr. Willyard – Leffer	\$500
c. Dr. Campbell – Gaiser	\$500
d. Dr. Campbell – Bell	\$500

\$2,000**4. JOURNAL**

a. Volume 68 Printing Cost	\$2,812.75
b. Journal Mailing Cost	\$131.33
c. Journal Editorial Cost	\$0.00

\$2,944.08**5. MISCELLANEOUS EXPENSES**

1. Poster Boards	\$173.96
2. Dr. Willyard, Plaque	\$87
3. Web-host.ipg, Dr. Itza	\$233.22

\$494.18

6. TRANSFER TO CD from Checking \$0.00

7. MEETING EXPENSES \$0.00

TOTAL EXPENSES \$7,438.26

Business meeting report

ARKANSAS ACADEMY OF SCIENCE COST OF JOURNAL

VOLUME	COPIES	PAGES	PRINTER CHARGE	TOT. VOL. COST	COST/ COPY	COST/ PAGE
35 (1981)	450	96	\$3,694.68	\$4,620.99	\$10.27	\$48.14
36 (1982)	450	110	\$5,233.28	\$5,291.69	\$11.76	\$48.11
37 (1983)	450	103	\$5,326.91	\$5,944.44	\$13.21	\$57.71
38 (1984)	450	97	\$5,562.97	\$6,167.72	\$13.71	\$63.58
39 (1985)	450	150	\$7,856.20	\$8,463.51	\$18.81	\$56.42
40 (1986)	450	98	\$6,175.20	\$6,675.20	\$14.23	\$68.11
41 (1987)	450	116	\$7,122.79	\$7,811.25	\$17.36	\$67.34
42 (1988)	450*	116	\$7,210.79	\$7,710.15	\$17.13	\$66.47
43 (1989)	450*	119	\$8,057.24	\$8,557.24	\$19.02	\$71.91
44 (1990)	450*	136	\$9,298.64	\$9,798.64	\$21.77	\$72.05
45 (1991)	450*	136	\$9,397.07	\$9,929.32	\$22.06	\$73.01
46 (1992)	450*	116	\$9,478.56	\$10,000.56	\$22.22	\$86.21
47 (1993)	400	160	\$12,161.26	\$12,861.26	\$32.15	\$80.38
48 (1994)	450	270	\$17,562.46	\$18,262.46	\$40.58	\$67.63
49 (1995)	390	199	\$14,725.40	\$15,425.40	\$39.55	\$77.51
50 (1996)	345	158	\$11,950.00	\$12,640.75	\$36.64	\$80.00
51 (1997)	350	214	\$14,308.01	\$15,008.01	\$42.88	\$70.13
52 (1998)	350	144	\$12,490.59	\$13,190.59	\$37.69	\$91.60
53 (1999)	350	160	\$13,686.39	\$14,386.39	\$41.10	\$89.91
54 (2000)	350	160	\$14,149.07	\$14,849.07	\$42.43	\$92.81
55 (2001)	360	195	\$16,677.22	\$17,498.22	\$48.61	\$89.73
56 (2002)	350	257	\$18,201.93	\$19,001.93	\$54.29	\$73.94
57 (2003)	230	229	\$14,415.12	\$15,715.12	\$68.33	\$68.62
58 (2004)	210	144	\$7,875.76	\$9,175.76	\$43.99	\$63.72
59 (2005)	215	226	\$16,239.04	\$17,835.84	\$82.96	\$78.92
60 (2006)	220	204	\$11,348.06	\$12,934.30	\$58.79	\$63.40
61 (2007)	195	150	\$8,196.84	\$9,914.69	\$50.84	\$66.10
62 (2008)	220	166	\$2,865.00	\$2,967.49	\$13.49	\$17.88
63 (2009)	213	206	\$3,144.08	\$3,144.08	\$14.76	\$15.26
64 (2010)	232	158	\$2,713.54	\$2,764.30	\$11.91	\$17.50
65 (2011)	200	194	\$2,915.12	\$2,963.03	\$14.82	\$15.27
66 (2012)	200	216	\$3,087.91	\$3,180.29	\$15.90	\$14.72
67 (2013)	200	238	\$3,311.42	\$3,396.32	\$16.98	\$14.27
68 (2014)	180	192	\$2,812.75	\$2,944.08	\$16.36	\$15.33

The Total Volume Cost equals the printer's charge plus the other miscellaneous charges (e.g. Mailing Costs).

- On Volume 42 the Academy received 560 copies, but the printer did not charge us for the extra 110 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 43 the Academy received 523 copies, but the printer did not charge us for the extra 73 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 44 the Academy received 535 copies, but the printer did not charge us for the extra 85 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 45 the Academy received 594 copies, but the printer did not charge us for the extra 144 copies. For comparison purposes the calculated cost/copy is based on 450 copies.
- On Volume 46 the cost was greater than usual due to the high cost of a second reprinting of 54 copies by a different printer.

Business meeting report**APPENDIX A****2015 AAS PRESENTATION AWARD WINNERS (underlined)****UNDERGRADUATE ORAL PRESENTATION
AWARDS: CHEMISTRY & BIOCHEMISTRY****1st Place**

“Bond Valence = Bond Length Relationships for Carbon-Carbon and Carbon-Oxygen Bonds” by Conner Harris and Franklin D. Hardcastle, Arkansas Tech University.

2nd Place

“The Use of a Dinuclear Molybdenum Oxalate Quinolinium Salt as an Oxidizing Agent” by Jake G. Windley and Burt Hollandsworth, Harding University.

3rd Place

“Development of a Quantification Method for Gold Nanoparticles in Mass Spectrometry Imaging” by Gunnar A. Klemmer, Harding University.

**UNDERGRADUATE ORAL PRESENTATION
AWARDS: PHYSICS & ENGINEERING****1st Place**

“Wave Profile for Anti-Force Waves with Maximum Possible Currents” by M. Hemmati, R. Horn, W.C. Childs and A.K. Meredith, Arkansas Tech University.

2nd Place

“High Resolution Spectroscopic Studies of Earth’s Atmosphere” by Emily J. Valerio and Edmond W. Wilson, Harding University.

3rd Place

“Atmospheric and Solar Laboratory at Harding” by Kyung Yoon and Edmond W. Wilson, Harding University.

**GRADUATE/UNDERGRADUATE ORAL
PRESENTATIONS: BIOLOGY****1st Place**

“Natural Nutrient Sources in the Cache River Watershed, Arkansas” by Mary K. Kilmer, Nicole Poe, Shelby Chappell, and Jennifer L. Bouldin, Arkansas State University.

2nd Place

“Fruit Consumption Rates and Potential Seed Dispersal Species for the American Persimmon” by Jillian F. Del Sol, Charli N. Davis, Natalie Skinner, Mimi Rebein, and Matthew D. Moran, Hendrix College

3rd Place

“Expression Analysis of an Insect Glucose Oxidase Transgene in Tobacco” by Jessie Kitchens and Nathan Reyna, Ouachita Baptist University.

**GRADUATE ORAL PRESENTATION AWARDS:
PHYSICS & ENGINEERING****1st Place**

“Terrain Effects on Tornado’s Path Deviation” by Nawfal S. Ahmed and R. Panneer Selvam, University of Arkansas, Fayetteville.

2nd Place

“Electrodeposition of Copper Sulfide Rods and Whiskers” by Michael Jason Newell, Joshua Vangilder, Maqsood Ali Mughal, Kayla Wood, Shyam Thapa, and Robert Engelken, Arkansas State University.

3rd Place

“Control and Power Factor Analysis of a Solid State DC Transformer” by Hussain Sayed, Ahmed Zurfi, and Jing Zhang, University of Arkansas at Little Rock.

POSTER PRESENTATION RECOGNITION

“Analysis Of A Ribosomal Protein Gene In Tumor Development” by Seth St. John, Wray Devon, Helen Beneš, and Mary Stewart, University of Arkansas at Monticello.

“Gene Discovery For Bioactive Phenylpropanoids In Echinacea Species” by Lauren Clai, E. Morehead, Jordyn Radke, and Stephen Grace, Southern Arkansas University.

“Screening Of Flathead Catfish For Heavy Metals In Ouachita River, AR.” by Casey C. O’Hara and Gija Geme, Southern Arkansas University.

Business meeting report

**APPENDIX B
RESOLUTIONS**

**Arkansas Academy of Science
99th Annual Meeting, 2015 Resolutions**

Be it resolved that we, the membership of the Arkansas Academy of Science (AAS) offer our sincere appreciation to Henderson State University for hosting the 99th annual meeting of the Academy. We thank the local arrangements committee: Martin Campbell (chair), David Bateman (co-chair), Jess Kelly, and Ingo Sehrans.

We sincerely thank Henderson State University for providing its facilities and service during the meeting and Airmark for the catering service.

We especially thank our keynote speaker, Mark A. Williamson for his inspiring talk entitled "Why Research?"

The Academy recognizes the important role of our session chairs: Cynthia Fuller (Ecology and Genetics), Ann Willyard (Morphology and Field Notes), Bradley Rowland (Theoretical Chemistry & Synthesis), Vincent Dunlap (Analytical Chemistry and Biochemistry), Rick McDaniels (Physics and Engineering), Troy Bray (Biology Notes and Ecology), Ed Wilson (Physics and Computer Science), Tommy Finley (posters), Anneq Ahmed, Travis Langley, Emilie Beltzer, and Rafael Bejanaro (Psychology).

Even greater appreciation and sincere gratitude extends to our dedicated judges for the student presentations including Jess Kelly (OBU); Bradley Rowland (HSU), James Engman (HSU), Matthew Moran (Hendrix), Mostafa Hemmati (ATU), and Mariusz Gajewski (ATU).

We congratulate our faculty and student researchers who presented papers and posters, whose efforts contribute directly to the future success of the Academy and the improvement and advancement of science in Arkansas.

The Academy recognizes its leadership and offers its thanks to this year's set of executive officers including Abdel Bachri (President), Ann Willyard (President Elect), Jeff Robertson (Past President), Ed Wilson (Vice President), Mostafa Hemmati (Treasurer and Journal Editor-in-Chief), Ivan Still (Journal Managing Editor), Panneer Selvam (Newsletter Editor), Salomon Itza (Webmaster), Collis Geren (Historian), and Jeff Robertson (Secretary).

Respectfully submitted on this 11th day of April, 2015.
Resolutions Committee: Abdel Bachri (President), and Martin Campbell (chair of local organizing committee)

Business meeting report

2014/2015 MEMBERSHIP

LIFE MEMBERS

FIRST MI.	LAST NAME	INSTITUTION
Edmond J.	Bacon	University of Arkansas-Monticello
Vernon	Bates	Ouachita Mountains
Floyd	Beckford	Lyon College
Don	Bragg	USDA Forest Service
Wilfred J.	Braithwaite	University of Arkansas-Little Rock
Dan	Bullock	Arkansas Tech University
Calvin	Cotton	Geographics Silk Screening Co.
Betty	Crump	Ouachita National Forest
James	Daly	UAMS (retired)
Leo	Davis	Southern Arkansas University
Mark	Draganjac	Arkansas State University
Jim	Edson	University of Arkansas-Monticello
Kim	Fifer	UAMS
James H.	Fribourgh	University of Arkansas-Little Rock
Collis	Geren	University of Arkansas
John	Giese	Ark. Dept. of Env. Qual. (ret)
Walter	Godwin	University of Arkansas-Monticello
Anthony	Grafton	Lyon College
Joe M.	Guenter	University of Arkansas-Monticello
Joyce	Hardin	Hendrix College
George	Harp	Arkansas State University
Phoebe	Harp	Arkansas State University
Gary	Heidt	University of Arkansas-Little Rock
Mostafa	Hemmati	Arkansas Tech University
Philip	Hyatt	Retired
Shahidul	Islam	University of Arkansas-Pine Bluff
Cynthia	Jacobs	Arkansas Tech University
Douglas	James	University of Arkansas
Ronald	Javitch	Natural History Rare Book Found.
Art	Johnson	Hendrix College
Cindy	Kane	UAMS
Tim	Kelly	Ouachita Baptist University
Scott	Kirkconnell	Arkansas Tech University
Roger	Koepe	University of Arkansas
Suresh	Kumar	University of Arkansas-Fayetteville
Roland	McDaniel	FTN Associates
Grover P.	Miller	UAMS
Herbert	Monoson	ASTA
Mansour	Mortazavi	University of Arkansas-Pine Bluff
James	Peck	University of Arkansas-Little Rock
Michael	Rapp	University of Central Arkansas
Dennis	Richardson	Quinnipiac College
Jeff	Robertson	Arkansas Tech University
Henry	Robison	Southern Arkansas University
Benjamin	Rowley	University of Central Arkansas
David	Saugey	U.S. Forest Service
Ivan	Still	Arkansas Tech University
Suresh	Thallapuranam	University of Arkansas-Fayetteville
Stanley	Trauth	Arkansas State University
Gary	Tucker	FTN Associates
Renn	Tumlison	Henderson State University
Scott	White	Southern Arkansas University
James	Wickliff	University of Arkansas
Robert	Wiley	University of Arkansas-Monticello
Steve	Zimmer	Arkansas Tech University

REGULAR MEMBERS

FIRST MI.	LAST NAME	INSTITUTION
Hashim	Ali	Arkansas State University
Glen	Akridge	Northwest Arkansas Community College
Jean	Aycock	University of Arkansas at Monticello
Abdel	Bachri	Southern Arkansas University
Brent	Baker	Arkansas Natural Heritage Commission
Coskun	Bayrak	University of Arkansas-Little Rock
Daniel	Berleant	University of Arkansas-Little Rock
Frank	Blume	John Brown University
Martin	Campbell	Henderson University
Stephen	Chordas III	Ohio State University
Rajib	Choudhury	Arkansas Tech University
Matthew	Connior	South Arkansas Community College
Magda	El-Shenawee	University of Arkansas-Fayetteville
Karen	Fawley	University of Arkansas-Monticello
Marvin	Fawley	University of Arkansas-Monticello
Huaxiang	Fu	University of Arkansas-Fayetteville
Mariusz	Gajewski	Arkansas Tech University
Steve	Gann	Arkansas Tech University
Gija	Geme	Southern Arkansas University
Frank	Hahn	Philander Smith College
Franklin	Hardcastle	Arkansas Tech University
Laurence	Hardy	Museum of Life Science
John	Hunt	University of Arkansas at Monticello
Anahita	Izadyar	Arkansas State University
Salomon	Itza	University of the Ozarks
David	Jamieson	Crowder College
Daniel	Kennefick	University of Arkansas-Fayetteville
Brenda	Lauffart	Arkansas Tech University
David	Martinez	U.S. Fish and Wildlife Service
Chris	McAllister	Eastern Oklahoma State College-Idabel
Lloyd	Moyo	Henderson State University
Jim	Musser	Arkansas Tech University
Lawrence	Mwasisi	University of Arkansas-Pine Bluff
Henry	North	Harding University
Derrick	Oosterhuis	University of Arkansas-Fayetteville
Frederick	Paillet	University of Arkansas-Fayetteville
David	Paul	University of Arkansas-Fayetteville
Joshua	Sakon	University of Arkansas-Fayetteville
Blake	Sasse	Arkansas Game and Fish
Panneer	Selvam	University of Arkansas-Fayetteville
Kimberly	Smith	University of Arkansas
Richard	Standage	Ouachita National Forest
Mary	Stewart	University of Arkansas-Monticello
Ryan	Stork	Harding University
Abraham	Tucker	Southern Arkansas University
Brian	Wagner	Arkansas Game and Fish
Azida	Walker	University of Central Arkansas
J.D.	Willson	University of Arkansas-Fayetteville
Ann	Willyard	Hendrix College
Cathy	Wissehr	University of Arkansas-Fayetteville
Tsunezui	Yamashita	Arkansas Tech University
Jessica	Young	Arkansas Tech University
Yijun	Yu	Philander Smith College
Douglas	Zollner	The Nature Conservancy

Arkansas Academy of Science

SPONSORING/SUSTAINING MEMBERS

<u>FIRST MI.</u>	<u>LAST NAME</u>	<u>INSTITUTION</u>
Philip	Crandall	University of Arkansas-Fayetteville
Tim	Knight	Ouachita Baptist University
Gerhard	Mensch	American Innovations Academy
Ed	Wilson	Harding University

STUDENT and ASSOCIATE MEMBERS

<u>FIRST MI.</u>	<u>LAST NAME</u>	<u>INSTITUTION</u>
Selby	Chappell	Arkansas State University
Olivia	Jamieson	
Michael	Kee	Arkansas School for Mathematics, Sciences, and the Arts
Molly	Kennon	Arkansas State University
Mary	Kilmer	Arkansas State University
Gunnar	Klemmer	Harding University
Brandy	Kluthe	University of Arkansas-Fayetteville
Maqsood	Mughal	Arkansas State University
Jackson	Petty	Harding University
Kelsey	Ray	Arkansas Tech University
Shannon	Wiley	
Jake	Windley	Harding University

MAJOR INSTITUTIONAL SPONSORS

The Arkansas Academy of Science is an essential component in the science, technology, engineering and math pipeline for Arkansas. As a coalition of Arkansas scientists, it provides a local vehicle for presentation and publication of early scientific accomplishments in Arkansas. By promoting the work of Arkansas students, the Academy increases collaboration among the scientific community and provides a comprehensive network for scientific academics. These endeavors promote a higher standard of education within Arkansas and will encourage and promote a higher quality of life through educational opportunities.

As an integral part of the development and promotion of the Academy's mission, we wish to recognize the commitment and continued support of our Institutional Sponsors, The Arkansas Natural Heritage Commission and the Ouachita National Forest.

ARKANSAS NATURAL HERITAGE COMMISSION



Since 1973, the Arkansas Natural Heritage Commission (ANHC) has been working to conserve Arkansas's natural landscape. ANHC conducts research to determine which elements (species and natural communities) are most in need of protection. Field inventory documents the locations of elements of conservation concern. Information is also gathered from other sources, such as herbarium and museum collection records, and scientific publications such as the *Journal of the Arkansas Academy of Science*. ANHC's current strategic planning goals include working to expand the ecological literacy of Arkansans. The Arkansas Academy of Science is a critical partner in helping to address this goal and, in the long term, protect the natural heritage of our state. For more information about the ANHC research, inventory and protection efforts, including the System of Natural Areas around the state, visit the agency website at www.naturalheritage.com. Here is a link to the current newsletter featuring our support info as well. <http://www.naturalheritage.com/enews/archive.aspx?mid=13361>.

OUACHITA NATIONAL FOREST



Stretching from near the center of Arkansas to southeast Oklahoma, the pristine 1.8 million acre Ouachita National Forest is the South's oldest national forest, established on December 18, 1907 by President Theodore Roosevelt. Rich in history, the rugged Ouachita Mountains were first explored in 1541, by Hernando DeSoto's party of Spaniards. French explorers followed, flavoring the region with names like Fourche la Pave River. "Ouachita" is the French spelling of the Native American word "Washita" which means "good hunting grounds." The Forest's ecosystem management policy guarantees its management regime as an ecological approach, based upon the most current knowledge and best science, for providing multiple benefits from the Forest and encouraging careful use of the forest for the future. The research local to Arkansas and the Forest published by the *Journal of the Arkansas Academy of Science* is critical to informing and supporting appropriate management decisions, environmental assessments and biological evaluations. The Ouachita National Forest extends support of the Academy's efforts through this sponsorship.

For more information about the Forest, visit our webpage at: <http://www.fs.fed.us/r8/ouachita>.

A TRIBUTE TO Dr. COLLIS GEREN



Collis Geren, Ph.D. retired from the University of Arkansas, Fayetteville, in June of 2010 as the longtime Dean of the Graduate School and Vice Provost of Research and Sponsored Programs. He has been a longtime supporter and member of the Arkansas Academy of Science, with quality statewide science education as a top personal priority.

He was appointed Associate Vice Chancellor for Research and Dean of the Graduate School in 1991. At that time, the Graduate School had fewer than 2,400 students. By the fall of 2008, the Graduate School enrollment had climbed to 3,370, and new awards for research had grown from \$41.2 million in 1997 to \$72.3 million in 2006.

Collis' first priority has always been the students. He started his career with a B.S.E. from Northeastern State College in Tahlequah, OK in 1967. That was followed by three years in a job he loved, as the high school science teacher in Picher, Oklahoma. Higher education beckoned and that led to an M.S. in chemistry from Kansas State College of Pittsburg and a Ph.D. in biochemistry at Oklahoma State University studying the biological activity of the venom of the brown recluse spider, *Loxosceles reclusa*. During this time, he learned to "love" spider hunting, and he would collect as many as 200 specimens a night in dark, old barns and buildings. After two years as a Research Associate at the University of Kansas Medical Center studying enzymes, in 1976 he accepted a position as Assistant Professor of Chemistry and Biochemistry at the University of Arkansas, Fayetteville. At the U of A, his research interests returned to the biological and chemical characterization of venom components of the brown recluse spider and expanded to include the venom of some snakes such as rattlesnakes and copperheads. A year later, the National Institute of Environmental Health Sciences made him the first Arkansas recipient of a Research Career Development Award with an \$110,000 research grant. The grant paid his salary and benefits for five years while he researched the toxic components of venoms. In 1986, the Arkansas Alumni Association recognized him with its Distinguished Alumni Award for teaching and research. In the course of collecting thousands of spiders followed by the removal of microscopic venom glands, protein purification and characterization techniques, monoclonal antibody technology, and biochemical pharmacology, he supervised 9 Ph.D. students, 2 M.S. students and 9 Honors Undergraduates. Also during his teaching career in Chemistry and Biochemistry, he authored or co-authored proposals that resulted in more than \$7.5 million in funding for research and more than 50 publications.

Arkansas Academy of Science

Collis' titles have changed through the years. He was promoted to Associate Professor in 1979 and Professor in 1984. He was president of the honorary science fraternity Sigma Xi from 1983-84. In 1986 Collis became Vice-chair of Chemistry and Biochemistry and served as Chair from 1987-91. He also served as the interim first Chair of the newly created Department of Biological Sciences Department from 1990--91. Collis was elected Chair of the Campus Faculty in 1991. In December 1991, he became Dean of the Graduate School and Associate Vice Chancellor for Research. In 2000 the last title changed to Vice Provost of Research and Sponsored Programs and Dean of the Graduate School.

Collis has served and continues to serve in a variety of statewide committees some of which are: Arkansas EPSCOR Committee (1987-2010) ; SILO Board; NASA Space Grant Committee; Game & Fish Commissioner (2/91-6/91); State SREB/non SREB Coordinator (11/90-6/93); EPSCoR Committee Co-Chair (1992-93); EPSCoR Committee Chair (1993-2000); EPSCOR Committee Vice Chair, 2000-2010; DOE EPSCoR Chair; EPA EPSCoR Subcommittee (1991-2010); NASA EPSCoR (1993-2010); DoD EPSCoR Chair for Arkansas (1993-2010) Governor's Task Force for Manufacturing Extension (1993-2010); Governor's R&D Task Force; Clinton Library Academic Program Committee; ORAU University, Government and Industry Relations Committee (1991-1995). He has also been involved in ASTA, STEM, and the Arkansas Discovery Museum Board.

As Dean of the Graduate School, Collis was responsible for the quality of the graduate academic programs as well as the quality and quantity of graduate students. The University of Arkansas Press, the Survey Research Center, the Arkansas Biotechnology Center, and the interdisciplinary Ph.D. Programs in Photonics and Electronic Materials, Public Policy, and Cell and Molecular Biology all reported to Collis.

As Vice Provost, some of his duties involved assisting university personnel in obtaining funding for their scholarship, then initiating the management once awards were made. He was also responsible for the management of the University's intellectual property and a deactivated nuclear reactor (SEFOR) owned by the UofA. Since retirement, Collis hasn't slowed down. He is a lifelong car enthusiast and retirement has allowed him more time to focus on his love of restoring vintage automobiles. Collis is also enjoying being a grandfather to two young grandsons, and he has found himself an active participant in his wife, Lois', orchid hobby (obsession). Lois and Collis are also active fishermen, and Collis continues to enjoy completing home remodeling projects. He is currently collaborating on a book, and remains a steadfast supporter of the Arkansas Academy of Science.

KEYNOTE ADDRESS

“Why Research?”

By Mark A. Williamson, PhD Geochemist
Geochemist, Geochemical Solutions, LLC. Environment, Forensics, Engineering



Research has been described as the scholarly pursuit of new knowledge, discovery, or creative activity in an area with the goal of advancing that area's frontiers or boundaries. The foremost reason for conducting research can be as varied as the people who are asked. However, there are several over-arching reasons that lay the foundation of why we research. Among these are the joy of direct experience, personal growth, search for beauty or truth, and a commitment to reason in problem solving. To be human is to pursue these ends and is the answer to the question “Why research?”

Dr. Williamson is an environmental geochemist with over 25 years of experiencing in consulting, basic/applied research and educational settings. Mark has worked extensively with the mining and associated industries and has been involved in geochemical studies and site evaluations across the United States as well as the Philippines, Peru, Australia, Indonesia, Argentina, Canada and Magnolia. Dr. Williamson has substantial experience with acid rock drainage (ARD), which began 25 years ago with his Ph.D. graduate studies in the kinetics of pyrite oxidation and sulfur geochemistry. His experience also includes characterization of mine material for potential ARD formation using industry standard methods, prediction of water quality from mine facilities, support for engineered construction design of mine waste facilities, pit lake evaluations, and water treatment design support. In addition to ARD, Mark has conducted studies involved with metals in aquatic and terrestrial environments, geochemical engineering, and the fate and transport of chemicals in the environment. He also has provided expert witness forensic support in a number of matters.

Arkansas Academy of Science

SECTION PROGRAMS ORAL PRESENTATIONS

ORAL SESSIONS: FRIDAY 2:00-5:00

BIOLOGY ECOLOGY AND GENETICS Reynolds 120
CHAIR: Cynthia Fuller Ph.D. Henderson State University

2:00 AVAILABLE FORAGE IN SHOREBIRD HABITAT IN SOUTHEAST ARKANSAS

Jean E. Aycock and Christopher G. Sims, Ph.D.
University of Arkansas at Monticello

2:20 NATURAL NUTRIENT SOURCES IN THE CACHE RIVER WATERSHED, ARKANSAS

Mary K. Kilmer, Nicole Poe, Shelby Chappell, and Jennifer L. Bouldin,
Ph.D. Arkansas State University

2:40 FRUIT CONSUMPTION RATES AND POTENTIAL SEED DISPERSAL SPECIES FOR THE AMERICAN PERSIMMON

Jillian F. Del Sol, Charli N. Davis, Natalie Skinner, Mimi Rebein, and
Matthew D. Moran, Ph.D. Hendrix College

3:00 ECOLOGY OF THE TRAPDOOR SPIDER, *MYRMEKIAPHILA* *COMSTOCKI*, IN THE OUACHITA MOUNTAINS OF ARKANSAS

Laurence M. Hardy, Ph.D. Ouachita Mountains Biological Station

BIOLOGY:MORPHOLOGY AND FIELD NOTES Reynolds 303
CHAIR: Ann Willyard, Ph.D. Hendrix University

2:00 THE PREVELANCE OF PATELLAR TENDONITIS IN MEN'S AND WOMEN'S SPORTS

Terance A. Carter and Margaret Tudor, Ph.D. Henderson State University

2:20 COCCIDIA (APICOMPLEXA: EIMERIIDAE) OF THREE-TOED BOX TURTLES, *TERRAPENE CAROLINA TRIUNGUIS* (REPTILIA: TESTUDINES), FROM ARKANSAS AND OKLAHOMA

C.T. McAllister¹, R.S. Seville², D. Motriuk-Smith², C. Hudson², M.B.
Connior³ and H.W. Robison⁴

¹Science and Mathematics Division, Eastern Oklahoma State College,
Idabel, OK 74745; ²Department of Zoology and Physiology, University of
Wyoming-Casper, Casper, WY 82601; ³Life Sciences, Northwest Arkansas
Community College, Bentonville, AR 72712; ⁴9717 Wild Mountain Road,
Sherwood, AR 72120

2:40 RED CROSSBILL INVASION OF NORTHWESTERN ARKANSAS DURING WINTER 2012-2013

Kimberly G. Smith and Joseph C. Neal. University of Arkansas

3:00 ATYPICAL HEAD MARKINGS OF THE OUACHITA MAP TURTLE IN THE UPPER OUACHITA RIVER OF ARKANSAS

Allison Surf and Renn Tumblison, Ph.D. Henderson State University

3:20 Break

3:50 TESTICULAR HISTOLOGY AND SPERM MORPHOMETRICS OF THE BIRD-VOICED TREEFROG, *HYLA AVIVOCA* (ANURA: HYLIDAE), FROM ARKANSAS

Stan E. Trauth¹, Chris S. Thigpen¹, and Matt B. Connior²

¹Arkansas State University; ²North Western Arkansas Community College

4:10 NEW AND NOTEWORTHY ADDITIONS OF VASCULAR PLANTS TO THE ARKANSAS FLORA

Tyler L. Childs, Sydney Grant, Tiffany Graves, Ethan Holicer, Seth
McBroom, Logan Thomas, Allen Leible, James Peck^{*} and Brett Serviss,
Ph.D. Henderson State University; ^{*}University of Arkansas at Little Rock

CHEMISTRY: THEORETICAL AND SYNTHESIS Reynolds 322
CHAIR: Bradley Rowland, Ph.D.

2:00 SEMI-EMPIRICAL QUANTUM CHEMICAL CALCULATIONS OF AL³⁺-BOUND AMINO ACID COMPLEXES MODELED FOR NEURODEGENERATIVE DISEASES

Steven Adams, Fontaine Talyor, Jacques Iragena, and Frank Hahn, Ph.D.
Philander Smith College

2:20 THICKNESS UNIFORMITY FOR PECVD SiN

Daniel Bullock, Ph.D. Arkansas Tech University

2:40 SYNTHESIS AND CHARACTERIZATION OF COPPER(II) COMPLEX WITH HEXADENTATE HEMI-CAGE LIGAND

Megan Fuller, Charles Mebi, and Anwar Bhuiyan, Ph.D.
Arkansas Tech University

3:00 BOND VALENCE-LENGTH RELATIONSHIPS FROM ATOMIC ORBITAL EXPONENTS

Franklin D. Hardcastle, Ph.D. Arkansas Tech University

3:20 Break

3:50 BOND VALENCE = BOND LENGTH RELATIONSHIPS FOR CARBON-CARBON AND CARBON-OXYGEN BONDS

Conner Harris and Franklin D. Hardcastle, Ph.D. Arkansas Tech University

4:10 ELECTRODEPOSITION OF COPPER SULFIDE RODS AND WHISKERS

Michael Jason Newell, Joshua Vangilder, Maqsood Ali Mughal, Kayla
Wood, Shyam Thapa, and Robert Engelken, Ph.D.
Arkansas State University

4:30 THE USE OF A DINUCLEAR MOLYBDENUM OXALATE QUINOLINIUM SALT AS AN OXIDIZING AGENT

Jake G. Windley and Burt Hollandsworth, Ph.D. Harding University

CHEMISTRY: ANALYTICAL AND BIOCHEMISTRY Reynolds 330
CHAIR: Vincent Dunlap, Ph.D. Henderson State University

2:00 DESIGN OF TAUTOMERICALLY AMBIGUOUS CYTOSINE- BASED NUCLEOSIDES AS POTENTIAL ANTI-HIV AGENTS

Duy Ha, Chase Elkin, and Vincent K. Dunlap, Ph.D.
Henderson State University

Meeting Report

- 2:20**
RNA PHOSPHORAMIDITE MONOMER SYNTHESIS: AN EXAMINATION OF PHOSPHITE SELECTIVITY IMPROVEMENT
Sarah Holt, Kyle Harvey, and Vincent K. Dunlap, Ph.D.
Henderson State University
- 2:40**
DEVELOPMENT OF A QUANTIFICATION METHOD FOR GOLD NANOPARTICLES IN MASS SPECTROMETRY IMAGING
Gunnar A. Klemmer, Harding University
- 3:00**
ANALYSIS OF TWO BASE-RING WARE II JUGLETS FROM THE LATE BRONZE AGE FOR OPIOID DERIVATIVES VIA GC-MS
Jackson R. Petty and Dennis Province, Ph.D. Harding University
- 3:20** **Break**
- 3:50**
DEVELOPMENT OF BREATH ANALYSIS METHODS
Maegen L. Sloan and Edmond W. Wilson, Ph.D. Harding University
- 4:10**
BALLOONSAT: HIGH ALTITUDE MEASUREMENT OF METHANE CONCENTRATION
Jennifer R. Sullivan, Bryant Fong, and Tillman Kennon, Ph.D.
Arkansas State University
- 4:30**
DETERMINATION OF MERCURY IN LIVING AND NON-LIVING SYSTEMS
James Lowe, Dylan Campbell, David May, Allie Davis, Allison Surf, T. David Bateman, Ph.D. and Renn Tumblison, Ph.D.
Henderson State University
- PHYSICS AND ENGINEERING** **Reynolds 127**
CHAIR: Rick McDaniels, Ph.D. **Henderson State University**
- 2:00**
TERRAIN EFFECTS ON TORNADO'S PATH DEVIATION
Nawfal S. Ahmed and R. Panneer Selvam, Ph.D.
University of Arkansas, Fayetteville
- 2:20**
WAVE PROFILE FOR ANTI-FORCE WAVES WITH MAXIMUM POSSIBLE CURRENTS
M. Hemmati, R. Horn, W.C. Childs and A.K. Meredith
Arkansas Tech University
- 2:40**
CALCULUS PATHOLOGIES
Shomari Hunter and Duane Jackson, Ph.D. Henderson State University
- 3:00**
GENERATION OF INCE-GAUSSIAN LASER BEAMS
Kelsey D. Ray and Jessica Young, Ph.D. Arkansas Tech University
- 3:20** **Break**
- 3:50**
CONTROL AND POWER FACTOR ANALYSIS OF A SOLID STATE DC TRANSFORMER
Hussain Sayed, Ahmed Zurf, and Jing Zhang, Ph.D.
University of Arkansas at Little Rock
- 4:10**
HIGH RESOLUTION SPECTROSCOPIC STUDIES OF EARTH'S ATMOSPHERE
Emily J. Valerio and Edmond W. Wilson, Ph.D. Harding University
- 4:30**
ATMOSPHERIC AND SOLAR LABORATORY AT HARDING
Kyung Yoon and Edmond W. Wilson, Ph.D. Harding University
- PSYCHOLOGY** **Garrison: Galloway**
CHAIR: Anneq Ahmed, Ph.D. **Henderson State University**
- 2:00** **BLURRED FAMILY LINES: HOW SURROGATE PARENTING STYLES AFFECT CHILDREN THROUGHOUT THEIR LIVES**
Matt D. Baldwin and Travis Langley, Ph.D. Henderson State University
- 2:20** **THE myGAZE® EYE TRACKER: A VISIONARY DEVICE FOR A VISIONARY EXPERIMENTER**
Logan Elmore and Emilie Beltzer, Ph.D. Henderson State University
- 2:40** **IS IT BETTER TO BE LOVED OR FEARED?**
Brittany A. Freeman and Travis Langley, Ph.D.
Henderson State University
- 3:00**
THE EFFECT OF COMPUTER-BASED, INTERACTIVE MODULES ON STUDENT LEARNING OF DIFFUSION
Rachel M. Rowland, J. Steve Oliver, Georgia W. Hodges, and Janet Lanza, Ph.D. University of Georgia
- 3:20** **Break**
- 3:50**
CAUSES AND CONSEQUENCES OF RIVALRY: HOW DO RIVALS SEE EACH OTHER?
Steven H. Jacobs and Travis Langley, Ph.D. Henderson State University
- PSYCHOLOGY II** **Garrison: Wilson**
CHAIR: Travis Langley, Ph.D. **Henderson State University**
- 2:00**
WHAT'S WRONG WITH HARLEY QUINN? SYSTEMATICALLY ELIMINATING POTENTIAL DIAGNOSES
Ashley R. Bles and Travis Langley, Ph.D. Henderson State University
- 2:20**
RED HOOD VIGILANTISM: MANHOOD, MOTIVATION, AND MURDER
Dillon C. Hall and Travis Langley, Ph.D. Henderson State University
- 2:40**
MOTIVES AND METHODS OF MACHIAVELLIAN MANIPULATION IN "BATMAN: HUSH"
Coley R. Henson and Travis Langley, Ph.D. Henderson State University
- 3:00**
BEYOND BATGIRL: POSTTRAUMATIC RECOVERY AND COPING MECHANISMS
Brian C. Maulden and Travis Langley, Ph.D. Henderson State University
- 3:20** **Break**
- 3:50**
THE DARK KNIGHT SHINES: PRACTICAL VS POSITIVE MOTIVES FOR HELPING OTHERS MEET THEIR POTENTIAL
O'Dell R. Perry-Johnson and Travis Langley, Ph.D. Henderson State University
- 4:10**
RELATIONSHIPS BETWEEN ASOCIALITY AND MANIPULATIVENESS: CAN YOU MANIPULATE PEOPLE WITHOUT UNDERSTANDING THEM?
Darian N. Sisson and Travis Langley, Ph.D. Henderson State University

Arkansas Academy of Science

ORAL SESSIONS: SATURDAY 8:30-12:00

BIOLOGY: NOTES AND ECOLOGY Reynolds 120
CHAIR: Troy Bray, Ph.D. Henderson State University

8:30
GENETIC ANALYSIS OF BACTERIA FROM CAVE CRICKETS FROM BLANCHARD SPRINGS CAVERNS, ARKANSAS

Leah Efird, Itzela Cruz, Caitlyn Gosch, Taylor Lee, Charlotte Wetzlar, and James Engman, Ph.D. Henderson State University

8:50
THE ARKANSAS ENDEMIC FLORA AND FAUNA: AN UPDATE WITH ADDITIONAL SPECIES

H.W. Robison¹, and C.T. McAllister²
¹9717 Wild Mountain Drive, Sherwood, AR 72120, ²Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

9:10
TREE MORTICULTURE TO PRODUCE FOOD PLOTS FOR WOODPECKERS

Seth W. Pearson and Robin M. Verble-Pearson, Ph.D.
 Texas Tech University

9:30 Break

9:50
RECENT HISTORY OF MOUNTAIN LION (*PUMA CONCOLOR*) OBSERVATIONS IN ARKANSAS, WITH NOTES ON THE INDIVIDUAL KILLED IN BRADLEY COUNTY, ARKANSAS IN 2014

Renn Tumilson¹ and Mark Barbee².
¹Henderson State University; ²Arkansas Game and Fish Commission, Monticello, AR 71655

10:10
NEW RECORDS AND OBSERVATIONS OF THE AMERICAN BADGER (*TAXIDEA TAXUS*) IN ARKANSAS

Renn Tumilson¹ and Blake Sasse²
¹Henderson State University; ²Arkansas Game and Fish Commission, Mayflower, AR 72106

10:30
VERTEBRATE NATURAL HISTORY NOTES FROM ARKANSAS, 2015

R. Tumilson¹, M.B. Connior², H.W. Robison³, C.T. McAllister⁴, L.A. Durden⁵, D.B. Sasse⁶, and D.A. Saugey⁷
¹Henderson State University, ²South Arkansas Community College, El Dorado, AR 71730, ³9717 Wild Mountain Drive, Sherwood, AR 7212, ⁴Eastern Oklahoma State College, Idabel, OK 7474, ⁵Department of Biology, Georgia Southern University, Statesboro, GA 30458, ⁶Arkansas Game and Fish Commission, Mayflower, AR 72106, ⁷Nightwing Consulting, Jessieville, AR 71949

10:50
FIRE IN OZARK OAK FORESTS

Robin M. Verble-Pearson, Ph.D. Texas Tech University

CHEMISTRY Reynolds 330
CHAIR: Bradley Rowland, PhD Henderson State University

8:30
SOLUBILITY STUDIES OF TITAN'S HYDROCARBON LAKES
 Malissa M. Hoehn, Brandon M. Daughety, and Edmond W. Wilson, Ph.D. Harding University

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TITAN: A MODEL FOR EARLY EARTH?

Connor D. Purvis and Edmond W. Wilson, Ph.D. Harding University

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PHYSIOLOGICAL EFFECTS OF MASSAGE THERAPY IN COLLEGE STUDENTS AND THE ELDERLY

Hunter Wayland, Christine Dickson, Anneq Ahmad, Ph.D., T. David Bateman, Ph.D. Henderson State University

9:30 Break

9:50
ANALYSIS OF BYPRODUCTS OF *LACTOBACILLUS* AND YEAST SPECIES METABOLISM IN WATER KEFIR VIA GAS CHROMATOGRAPHY

Justin Hunn and Dennis Province, Ph.D. Harding University

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DEVELOPMENT OF HIGHER IMPULSE HYBRID ROCKET MOTORS

Rachel A. Beeman and Edmond W. Wilson, Ph.D. Harding University

PHYSICS AND COMPUTER SCIENCE Reynolds 322
CHAIR: Edmond Wilson, Ph.D. Harding University

8:30
ROBOTIC ARM FOR SPACE MISSIONS

Stephanie J. Inabnet and Edmond W. Wilson, Ph.D. Harding University

8:50
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 Yijun Yu. Philander Smith College

9:10
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Osman A. Martinez and Kevin R. Lewelling, Ph.D.
 University of Arkansas - Fort Smith

9:30 Break

9:50 **WIFI GUIDANCE OF THE MARS ROVER**

Daniel H. Schwartz and Kevin R. Lewelling, Ph.D.
 University of Arkansas - Fort Smith

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 Shelby V. Sorrells and Edmond W. Wilson, Ph.D. Harding University

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HIGH RESOLUTION SPECTROGRAPH DESIGN
 Brennan M. Thomason, Stephanie J. Inabnet, Tamara B. Thomason, and Edmond W. Wilson, Ph.D. Harding University

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Shorebird Foraging Habitat in Southeast Arkansas

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Running Title: Shorebird Foraging in SE Arkansas

Abstract

Approximately 500,000 shorebirds travel through the Mississippi Alluvial Valley (MAV) each spring and fall. During migration, the average 45 g shorebird needs to eat approximately 8 g of invertebrates per day. While shorebird stopover habitat guidelines for the MAV are based on an expert estimate of 2 g of invertebrates/m², this estimate has not been quantified in Arkansas. Invertebrate biomass available for shorebird foraging was examined on five properties in southeastern Arkansas during spring and fall migration (fall 2010, spring and fall 2011, and spring 2012).

Macroinvertebrate biomass was less than the estimated 2 g/m² in three of the four sampled seasons. Further validation of the expert invertebrate biomass estimate should be undertaken in the other regions of the MAV. These results suggest that current land management of shorebird stopover habitat in southeastern Arkansas is not providing adequate invertebrate forage to reach the current habitat management goals.

Introduction

Land managers in the Mississippi Alluvial Valley (MAV) face the challenge of providing stopover sites to approximately half a million shorebirds each fall and spring (Loesch et al. 2000). Migrating shorebirds forage on aquatic and benthic macroinvertebrates along with small amounts of terrestrial macroinvertebrates, small fish, and some plants (Lehnen and Kremetz 2007, Mitchell and Grubaugh 2005, Skagen and Oman 1996). Invertebrate abundance is considered to be more important to shorebirds than species composition because migrating shorebirds are highly flexible in their prey selection (Lehnen and Kremetz 2007, Mitchell and Grubaugh 2005, Skagen and Oman 1996). This flexibility in prey selection is due to the high variability of available habitat from year to year, requiring shorebirds to be adaptive in their response to

changing prey availability (Davis et al. 2005, Davis and Smith 1998, Mitchell and Grubaugh 2005).

Foraging habitat in the MAV is generally more abundant during spring migration than fall migration because of natural hydrology, flooding of rice fields, and spring rains (Loesch et al. 2000). Fall migration, however, occurs when seasonal precipitation is at its lowest, and when rice fields are drained to facilitate harvest. This dichotomy has led to the identification of the fall migration period as the time of most concern for shorebird stopover habitat management by Partners in Flight (PIF). PIF suggests that shorebird management objectives are most easily met on public lands that are currently managed for waterfowl (Loesch et al. 2000). Ensuring management compatibility among shorebirds, early migrant waterfowl, and late migrant waterfowl is of great concern (Loesch et al. 2000).

An average shorebird needs approximately 6 g of invertebrate forage daily in order to maintain its body mass (Loesch et al. 2000). An additional 2 g must be consumed daily to balance the increased energy requirements of migration. PIF used an expert estimate of 2 g of invertebrates/m² to calculate that the average migrating shorebird required 4 m² of foraging habitat per day (Loesch et al. 2000). Following these habitat need estimates, PIF recommended a total of 2000 ha of foraging habitat are required to support the estimated 500,000 shorebirds migrating through the MAV (Loesch et al. 2000).

Further research and validation is needed throughout the MAV. Little quantitative work has been done to validate the PIF estimate with regards to benthic communities and available biomass in the MAV (Augustin et al. 1999). The objective of this study is to determine whether public and private lands in SE Arkansas are meeting the PIF estimate of available invertebrate forage.

Materials and Methods

Each site was visited weekly during the sampling period. In fall 2010, sampling took place from 25 August to 13 October though the actual migration period began approximately 2 weeks earlier. In spring 2011, sampling took place from 24 March to 19 April. In fall 2011, sampling took place from 11 August to 29 September. Finally, in spring 2012, sampling took place from 13 March to 12 April (Table 1).

Four randomly selected substrate samples were collected at each site each week with a 10 cm diameter core sampler (Miller and Bingham 1987). Substrate samples 5 cm deep were collected to sample the depth of substrate available to most shorebirds (Piersma 1987, Sherfy et al. 2000). Two substrate samples were taken in the water < 10 cm in depth and two substrate samples were taken above the waterline on the mudflat. Since different species of shorebirds forage in different areas (for example, some forage only in the water, some forage only on mudflats, some forage on the waterline) this allowed for better coverage of the range of shorebird foraging habitat. Samples were preserved in the field with a 70% ethanol solution. Invertebrates were hand sorted then dried at 60 degrees Celsius for 24 hrs (Augustin et al. 1999, Sherfy et al. 2000). Samples were weighed to the nearest 0.001 g to establish available biomass (Augustin et al. 1999, Sherfy et al. 2000).

Fall 2011 invertebrate biomass was log-transformed in order to meet assumptions of homogeneity and normality (Augustin et al. 1999). Data from all other seasons met assumptions of homogeneity and normality. The one-sample, one sided Student's *t*-test was used to compare each site's mean invertebrate biomass to the PIF's 2 g/m² estimate (Loesch et al. 2000). Single factor ANOVAs were used to detect differences in mean invertebrate biomass both among sites in each season and weekly mean invertebrate biomass at each site in each season (Andrei et al. 2008). If weekly means were found to be different ($P < 0.05$), a Tukey's multiple comparison test was used (Augustin et al. 1999).

Sampling of all sites except Five Oaks took place during the spring and fall migration periods over two years. Five Oaks was sampled during spring 2011, fall 2011, and spring 2012. Each season's sampling began when migratory species began to be reported by observers on the eBird.org database, and ceased when no migratory species were observed at any study site (Sullivan et al. 2009).

Five management areas in southeastern Arkansas

were sampled. The Bob White Memorial Wetlands Research and Teaching Station (BWMW) is located in Chicot County, Arkansas. The property originally was used for agriculture, but was enrolled by the Natural Resources Conservation Services in 2002-2003 as a permanent Wetland Reserve Program easement (Whittsitt and Tappe 2009). Current vegetation includes cattails (*Typha* spp.), Eastern Baccharis (*Baccharis halimifolia*), and hardwood saplings. The study site at BWMW consisted of a 1.8 ha pond and was not actively managed.

Five Oaks is a private hunting club managed by Five Oaks Wildlife Services in Arkansas County, Arkansas. The study site consisted of a 5.2 ha impoundment, managed to mimic the natural hydrology of the area; flooding in winter and spring and slow drying through summer and fall. No vegetation was planted, and the site had minimal moist soil plants.

Overflow National Wildlife Refuge (Overflow NWR) is located in Ashley County, Arkansas. Overflow NWR covers approximately 5260 ha of wetlands consisting of bottomland hardwoods, seasonally flooded impoundments, and greentree reservoirs. The impoundments at Overflow NWR were alternately leased for agriculture and managed for shorebirds and waterfowl. Three impoundments were sampled over the course of this study due to changes in which impoundments were under waterfowl and shorebird management

Table 1. Timeline and sampling area size (ha) on each site during each migration season.

Site	Fall 2010	Spring 2011	Fall 2011	Spring 2012
BWMW	1.8	1.8	1.8	1.8
Five Oaks	X	5.2	5.2	5.2
Halowell	0.6	8.5	8.5	8.5
Overflow NWR	53.3	6.0	6.0	11.6
Wrape	15.6	15.6	15.6	15.6

Results

Only BWMW and Halowell Reservoir in fall 2010, and Overflow NWR and the Wrape Plantation in spring 2012 had invertebrate biomasses that were not less than the PIF estimate of 2 g/m². Of the overall season mean invertebrate biomass, only fall 2010 was

Shorebird Foraging in SE Arkansas

not less than the 2 g/m² estimate (Table 2). During fall 2010 and fall 2011, no invertebrates were collected at Overflow NWR because the impoundment was completely dry.

No difference in invertebrate biomass was detected among sites in fall 2010 ($P = 0.7383$), spring 2011 ($P = 0.4289$), or spring 2012 ($P = 0.0792$). Invertebrate biomass at BMWW was over three times greater than any of the other sites in fall 2011 ($P = 0.0042$).

In all seasons, only BMWW and Halowell Reservoir in fall 2010, and Overflow NWR and the Wrape Plantation in spring 2012 were not less than the PIF estimate of 2 g/m². Of the overall season mean invertebrate biomass, only fall 2010 was not less than the 2 g/m² estimate (Table 2).

Table 2. Mean invertebrate biomass of each site during each season, and overall mean season invertebrate biomass in g/m² ± SE. T-test p-value results testing for biomass ≥ 2.0 g.

Site	Mean Invertebrate Biomass	P-Value
Fall 2010		
<i>Overall</i>	1.45 ± 0.37	($P = 0.0685$)
BMWW	1.50 ± 0.61	($P = 0.2105$)
Five Oaks	X	X
Halowell	1.84 ± 0.79	($P = 0.4216$)
Overflow NWR	X	X
Wrape	1.05 ± 0.50	($P = 0.0342$)
Spring 2011		
<i>Overall</i>	0.98 ± 0.14	($P < 0.0001$)
BMWW	0.39 ± 0.09	($P < 0.0001$)
Five Oaks	1.17 ± 0.46	($P = 0.0093$)
Halowell	0.41 ± 0.12	($P < 0.0001$)
Overflow NWR	0.85 ± 0.37	($P = 0.0020$)
Wrape	1.13 ± 0.57	($P = 0.0317$)
Fall 2011		
<i>Overall</i>	0.79 ± 0.27	($P < 0.0001$)
BMWW	2.78 ± 0.91	($P = 0.0520$)
Five Oaks	0.78 ± 0.39	($P < 0.0001$)
Halowell	0.18 ± 0.09	($P < 0.0001$)
Overflow NWR	X	X
Wrape	0.16 ± 0.11	($P < 0.0001$)
Spring 2012		
<i>Overall</i>	0.99 ± 0.22	($P < 0.0001$)
BMWW	0.16 ± 0.11	($P < 0.0001$)
Five Oaks	0.18 ± 0.02	($P < 0.0001$)
Halowell	1.13 ± 0.35	($P = 0.0224$)
Overflow NWR	1.59 ± 0.27	($P = 0.1438$)
Wrape	1.87 ± 0.99	($P = 0.8981$)

Discussion

In three of the four sampling seasons, the average available invertebrate biomass was less than the 2 g/m² Partners in Flight (PIF) recommendation. Using the average invertebrate biomass of both fall seasons (1.18 g/m²), the estimate of needed shorebird foraging habitat in Arkansas increases from 520 ha to 881 ha, a 69.5% increase. However, Augustin et al. (1999) concluded that the invertebrate biomass of their study sites (2.15 to 5.74 g/m²) in western Tennessee were comparable to the PIF model requirements. Mitchell and Grubaugh (2005) found an average invertebrate biomass of 3.43 g/m² on their sites throughout the Lower MAV (Arkansas, Mississippi, and Louisiana), although the biomass ranged from less than 0.1 g/m² to 24.4 g/m².

In three of the four sampling seasons, the average available invertebrate biomass was less than the 2 g/m² PIF estimate (Augustin et al. 1999). The MAV covers approximately 10 million ha in seven states; using one estimate of highly variable factor such as invertebrate biomass to make habitat recommendations for the entire MAV may lead to overestimation of habitat needs in one area while underestimating needs in another (Smith et al. 1989).

Further validation of the PIF invertebrate biomass estimate should be undertaken in the other regions of the MAV. Whether the shortfalls found in this study were due to natural drought conditions, lack of funding for management activities, or the failure of waterfowl focused management to provide adequate fall stopover habitat for shorebirds, it is clear that habitat goals for southeastern Arkansas should be reassessed by Partners in Flight.

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Introduction of Florida Bass Alleles into Largemouth Bass Inhabiting Northeast Arkansas Stream Systems

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Running title: Introduction of Florida Bass Alleles into Largemouth Bass Inhabiting NE Arkansas Stream Systems

Abstract

Florida bass (*Micropterus floridanus*) have been introduced throughout much of the southern U.S. over the past 50 years. This species readily hybridizes with the extant largemouth bass (*M. salmoides*). Within Arkansas, the Florida bass is currently stocked in the southern half of the state. Previous studies of a northern Arkansas hatchery and a reservoir revealed the existence of Florida bass alleles in each. Other studies in Oklahoma and Texas have revealed the presence of Florida bass alleles in stream systems proximal to lakes stocked. Our goal was to investigate, using microsatellite analysis of 7 diagnostic loci, the presence of Florida bass alleles in 8 northeastern Arkansas streams to determine if Florida bass or hybrids had escaped from private farm ponds as compared to stocked reservoirs. We found rare instances of Florida bass alleles in most drainages, consistent with previous studies demonstrating a lack of containment of Florida bass once stocked. In Cane Creek, which flows adjacent to privately stocked farm ponds, one-third of the individuals had Florida bass alleles.

Introduction

State and federal agencies in the U.S. began stocking exotic fish species in the 1800's with the introduction of trout. Other sportfish species such as sunfishes and percids were eventually stocked into new and existing bodies of water across the U.S. The potential for negative consequences of these exotic stocking events were not initially considered, and their effects have forever changed the biological landscape of many North American watersheds.

One fish species regularly stocked over the past 50 years through much of the southern U.S. is the Florida bass (FB, *Micropterus floridanus*), which is stocked in reservoirs containing extant populations of Largemouth Bass (LMB, *Micropterus salmoides*). The FB has a

reputation of greater maximal growth than the LMB (Addison and Spencer 1971, Wright and Wigtil 1980, Horton and Gilliland 1993, Hobbs et al. 2002), with several state records of bass in southern US states comprised of exotic FB rather than native LMB (Oklahoma, Horton and Gilliland 1993; Texas, Lutz-Carrillo et al. 2006; Louisiana, Hughes and Wood 1995; and Arkansas, Lamothe and Johnson 2013). The Arkansas Game and Fish Commission (AGFC) stocks FB in the southern half of the state based upon thermal criteria established for stocking FB in Oklahoma (Gilliland 1992).

The native range of the FB is limited to peninsular Florida (Boschung and Mayden 2004), yet hybridization among FB and LMB occurred naturally in areas where the native ranges overlap. Hybridization also occurs readily in waters where FB are stocked in waters with extant LMB populations. Further, bass stocked in reservoirs can escape from whence they are stocked. Researchers have identified non-native Florida bass alleles in streams adjacent to reservoirs stocked with FB (Gelwick et al. 1995, Ray et al. 2012).

Additionally, private landowners often stock FB rather than LMB in Arkansas ponds, including in areas that are north of the limits of stocking practices by the AGFC. If individuals from that pond escape to a stream, FB alleles may be introduced. However, it is unlawful to release native or non-native aquatic wildlife into any waters of the state without the written permission of the Commission (AGFC 2014).

In an effort to determine whether FB were escaping from farm ponds into neighboring stream systems, bass were sampled from several streams (n = 8) in northeast Arkansas (Table 1). Northeast Arkansas was selected because only two waterbodies in this region, lakes Ashbaugh and Greenlee, have been historically stocked with FB by the AGFC. Lake Ashbaugh, was renovated with a fish kill using rotenone in 1996 and re-stocked with LMB (Johnson and Fulton 1999). Lake Greenlee was renovated in 2000 and stocked with FB thereafter. Streams were

Table 1. Fin clip samples collected from Largemouth bass and potential Florida bass in various locations in northeast Arkansas.

Location	No. Samples	Latitude, Longitude	Source	Stream Mouth
Martin's Creek near Ravenden	9	36.279306, -91.328056	Natural Spring	Spring River
Higginbotham Creek south of Jonesboro at Ingels Road	5	35.791972, -90.652806	Jonesboro City Drainage	Ditch Number 103
Des Arc Bayou near mouth at White River	3	35.009861, -91.520944	Ozark Mountains	White River
Bull Creek near Vinity Rd	23	35.074667, -91.829333	Ozark Mountains	Des Arc Bayou
Cane Creek near US 67/167	18	35.139833, -91.809111	Ozark Mountains	Des Arc Bayou
Spring River south of Hardy	5	36.292331, -91.438892	Mammoth Springs	Black River
Big Creek at AR Hwy 349	7	35.840617, -90.801826	Lake Frierson	Bayou DeView
Swan Pond Ditch near Raybourn Rd in Poinsett County	3	35.637518, -90.803378	Agricultural Drainage Ditch	Claypool Reservoir

chosen to represent a variety of smaller watersheds distant from reservoirs stocked with bass by the AGFC; each stream flows without dams or other obstructive barriers.

The objectives of this study were as follows: 1) to estimate the frequency of FB alleles in various northeastern Arkansas streams; and 2), if FB alleles are present, determine the level of intergradation of fish containing those alleles.

Materials and Methods

Sampling and DNA Processing

Fin clips were collected from angled fish and stored in 95% v/v ethanol solution. DNA extraction was performed using a modified version of the Saghai-Marooof *et al.* (1984) CTAB (chloroform tris-acetate-borate) method. The nucleic acid concentrations of the stock solutions were determined using a Thermo Scientific Nanodrop 8000c Spectrophotometer (Wilmington, DE) and standardized to working solution concentrations of 50 ng/μl.

The polymerase chain reaction (PCR) was performed using a modified version of the Lutz-

Carrillo *et al.* (2006) protocol. Seven microsatellite primer sets were divided into two multiplex reactions, MPX1 and MPX2, based on their annealing temperatures. MPX1 reactions consisted of the forward and reverse primers needed to amplify the loci *Lma12*, *Mdo7*, and *Msa21*. MPX2 reactions consisted of the forward and reverse primers to amplify *Mdo3*, *Mdo6*, *Msa13*, and *Msa29* (Colbourne *et al.* 1996, DeWoody *et al.* 2000, Malloy *et al.* 2000).

Forward primers of each set were tagged using fluorescent markers (Integrated DNA Technologies[®], Coralville, IA). Each multiplex reaction included 50 ng DNA, 0.4 uM each of upstream and downstream microsatellite primers, 0.2 mM of dNTPs, 1X REDTaq[®] PCR reaction buffer, and 0.5 U REDTaq[®] polymerase (Sigma-Aldrich, St. Louis, MO), and sterile water to a total volume of 10 μl. Each multiplex PCR reaction was performed using a Bio-Rad iCycler[®] Version 4.006 (Hercules, CA) with the conditions as follows: for MPX1, denature at 94 °C for 1.5 min, then 31 cycles of 94 °C for 30 s to denature, 47 °C for 30 s to anneal and 72 °C for 30 s for extension; for MPX2, denature at 94 °C for 1.5 min, then 32 cycles of 94 °C for 30 s to denature, 60 °C for 30 s to anneal and 72 °C

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for 1 min for extension.

The size of amplicons from the PCR reactions was determined by capillary electrophoresis using a Beckman-Coulter CEQ8000 system (Beckman-Coulter, Inc., Fullerton CA), and the CEQ fragment analysis software version 7.0. The results were then manually verified and recorded.

Statistical Analysis

Control samples from 3 Arkansas fish hatcheries (FB, Andrew H. Hulsey State Fish Hatchery, $n = 83$; LMB, Joe Hogan State Fish Hatchery, $n = 32$; and LMB, William H. Donham State Fish Hatchery, $n = 43$) were previously scored for each microsatellite locus (Allen et al. 2009); alleles were designated as exclusively LMB, exclusively FB, or shared between species. The program GeneAIEx (Peakall and Smouse 2006) was used to determine allelic frequencies for each locus for two bass populations, Cane Creek and Bull Creek, and for the entire data set.

The software program STRUCTURE 2.3 (Pritchard et al. 2000) was first used with an admixture model with correlated allele frequencies and default settings to establish pure species and their intergrades (50,000 burn-in steps; 500,000 Monte Carlo/Monte Carlo steps). The result of this analysis was a statistical value for the individual admixture proportion (q) of

each individual and for the population as a whole. Consistent with Allen et al. (2009), the number of clusters (k) was identified as 2, with values ranging from 0.0 (FLMB) to 1.0 (NLMB). The degree of admixture for Cane Creek ($n = 18$) and Bull Creek ($n = 23$) bass, the entire data set, and the hatchery controls were identified during a single run. The resultant output provided an individual admixture proportion (q) on a scale, 0.000 - 1.000, where 1.000 corresponds with LMB and 0.000 corresponds to FB for each individual. The q -value was used to classify individuals as to species following the 0.050 thresholds given by Schwartz and Beheregaray (2008). Pure LMB had q -values greater than or equal to 0.950, whereas pure FB had q -values less than or equal to 0.050. All broodstock controls were within this threshold and distinguished as pure species (NLMB: Joe Hogan Hatchery, $n = 32$; $q = 0.996$; William Donham Hatchery, $n = 42$; $q = 0.989$; FLMB: Andrew Hulsey Hatchery, $n = 83$; $q = 0.002$).

Results

Genetic Diversity

Over the entire data set, all 7 loci were polymorphic, though not for each population. The total number of alleles per locus ranged from 2 (Msa21) to

Table 2. Number of alleles per locus with allele sizes (base pairs) in parentheses for hatchery controls, the entire data set and Bull Creek and Cane Creek collection sites. The other sites are combined due to small sample sizes.

Locus	Andrew Hulsey $n = 83$	Joe Hogan $n = 32$	William Donham $n = 43$	Bull Creek $n = 23$	Cane Creek $n = 18$	Other stream samples $n = 32$	Total NE AR $n = 73$
<i>Lma12</i>	4	4	5	3	2	4	4 (103-121)
<i>Mdo7</i>	3	6	6	8	5	6	8 (164-183)
<i>Msa21</i>	5	1	1	1	2	2	2 (199-203)
<i>Mdo3</i>	4	9	5	7	7	9	10 (101-123)
<i>Mdo6</i>	2	2	2	2	2	4	4 (142-154)
<i>Msa13</i>	3	4	4	4	4	5	5 (188-196)
<i>Msa29</i>	3	5	6	5	6	8	9 (260-279)

10 (Mdo3), with a total of 42 alleles for all loci from the collected samples (Table 2). The fish from Bull Creek and Cane Creek had a total of 30 and 28 alleles for the 7 loci, respectively. The remaining stream samples had a total of 38 alleles and each of the 7 loci were polymorphic (Table 2). Cane Creek was polymorphic over all 7 loci, whereas Bull Creek was monomorphic at the Msa21 locus. The control samples from the Joe Hogan and William Donham Fish Hatcheries were monomorphic at the Msa21 locus, whereas the Andrew Hulseley Fish Hatchery samples were polymorphic at all 7 loci.

Nineteen alleles were classified as only FB alleles and another four alleles were designated as shared between FB and LMB. At the *Mdo3* and *Mdo6* loci, there were two alleles that were not found in any of the hatchery control samples, nor previously found in more than 5000 reservoir bass studied (Johnson *unpublished*).

Bass Genotypes

FB alleles were common in bass of Cane Creek ($x = 0.158$) and Des Arc ($x = 0.119$), yet were rare for other stream samples ($x = 0.032$; Table 3). Most stream bass populations had 1-2 percent FB alleles, although it must be reiterated that sample sizes are small. Trends were similar for alleles shared between species. Greater than one-fourth of the alleles for bass from Cane Creek were either exclusive to FB or shared between species. Most alleles contributing to the totals for other stream bass were shared between species rather than being exclusively FB alleles. Consistent with allele frequency data, bass from streams sampled had high average q -values, whereas Cane Creek had a lower q -value. The q -values of all bass ranged between 0.585 and 0.998, with an overall average q -value of 0.971 (Table 3). Of the 73 samples, there were only 7 fish that were not classified as pure LMB (q -value < 0.950). Six of these hybrid individuals were collected from Cane Creek, and had q -values ranging from 0.585 to 0.907. The other hybrid fish was collected from Des Arc Bayou and had a q -value of 0.932 (Table 3).

Table 3. Average allele frequencies and admixture proportions (q -values) and $1-q$ for bass from stream samples. The Mixed column represents alleles shared by both species.

Population	LMB	FB	Mixed	FB + Mixed	LMB q	FB $1-q$
Collection sites:						
Martins Creek	0.930	0.016	0.054	0.070	0.992	0.008
Higgin. Creek	0.875	0.018	0.107	0.125	0.991	0.009
Des Arc Bayou	0.810	0.119	0.071	0.190	0.970	0.030
Bull Creek	0.903	0.022	0.075	0.097	0.996	0.004
Cane Creek	0.737	0.158	0.105	0.263	0.907	0.093
Spring River	0.871	0.029	0.100	0.129	0.991	0.009
Big Creek	0.909	0.020	0.071	0.091	0.992	0.008
Swan Pond	0.977	0.000	0.023	0.023	0.998	0.002
Totals	0.876	0.048	0.076	0.124	0.971	0.029

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Discussion

Florida bass alleles were present, yet uncommon, in bass of northeast Arkansas stream systems other than for Cane Creek. The presence of FB alleles was unexpected, as the AGFC does not currently stock FB in most northeast Arkansas waters. The high incidence of FB alleles in Cane Creek is alarming. This creek flows proximal to several privately-owned farm ponds. One landowner adjacent to Cane Creek stated that he regularly pumps water into and out of the creek from his farm pond (*pers. commun.*). Further, overland flooding of this creek regularly occurs, creating linkages of pond and stream waters. Flooding events contributing to the contamination of bass stocks has been demonstrated in other systems (Maceina et al. 1988, Gelwick et al. 1995, Allen et al. 2009). Immigration of fish into streams would readily occur during those times. The nearest AGFC public lake stocked with FB is Lake Greenlee located east of Brinkley and is 65 km linearly from Cane Creek, and each is part of the White River Drainage. Lake Greenlee serves as a second potential source of FB alleles for bass in Cane Creek, yet has no connection to a stream system.

Several other studies have identified FB alleles in waters adjacent to locations where state agencies stock FB. For example, in Oklahoma Gelwick et al. (1995) used allozyme analysis at 5 diagnostic loci in order to determine the extent of introgression of FB in 21 stream populations through much of the state. Four percent of individuals sampled and 11% of the sites sampled showed the presence of FB alleles. They also found FB alleles in bass of the Arkansas River basin, which could have resulted in FB alleles being introduced to Arkansas bass; none of the streams of this study are part of the Arkansas River drainage system, however. Gelwick et al. (1995) noted that the highest concentration of FB alleles, up to 18% in one stream, was in southeastern Oklahoma where the Oklahoma Department of Wildlife Conservation regularly stocks FB. They also reported FB allele frequencies up to 2.5% in the northwest part of the state where there were historical stockings of FB. Similar to this study, they hypothesized that events of overland flooding could likely be the cause for FB introductions from farm ponds.

Ray et al. (2012) conducted a more focused study of bass populations in Texas streams using mitochondrial DNA analysis. They found high levels of fish (26%) possessing FB haplotypes. They identified FB alleles in their farthest sampling location

80 km away from the closest documented stocking site, indicating high dispersal potential of stocked bass and their progeny.

Johnson and Fulton (1999) noted FB allele persistence in Lake Ashbaugh of northeast Arkansas following regular stockings of LMB. Lake Ashbaugh was initially stocked with FB when the lake was constructed in 1981 and thereafter with LMB. Using allozyme analysis, Johnson and Fulton (1999) determined that 62% of the bass sampled from Lake Ashbaugh contained FB alleles and that roughly one-fourth of the alleles were FB alleles.

It is also possible that low levels of FB alleles are being unintentionally directly introduced throughout the state by the AGFC. Although the AGFC regularly screens their FB hatchery to maintain genetic purity of broodstock, they do not screen their LMB hatcheries for the presence of FB alleles. Historically, FB alleles were found in two LMB hatcheries (Hogan and Donham) by Johnson and Staley (2001), although, more recent analysis did not identify FB alleles in those hatcheries (Allen et al. 2009); researchers have identified contamination of FB broodstock prior to establishing genetic testing of those broodstock (Maceina et al. 1988, Gilliland and Whittaker 1989, Barthel et al. 2010). Periodic testing of native broodstock should be performed in order to reduce the chances of unintentionally introducing exotic alleles.

Lastly, anglers have been identified as transporting fish from one waterbody to another (Rahel 2004, 2010). Locally, in 2012, the AGFC had to disqualify what would have been the state record LMB (7.4 kg), a record held for 37 years, due to a lack of angler licensure. This bass was genetically confirmed using microsatellite analysis to be a FLMB in a reservoir not previously stocked with FLMB, but was within 2 km of a reservoir that had been stocked with FLMB (Lamothe and Johnson 2013).

The introduction of non-native FB into extant LMB populations offers the possibility of introducing maladaptive genes into those populations. These introductions could have long term consequences for generations due to the persistence of those alleles (Philipp 1991). Although outbreeding depression has not been observed in Arkansas bass populations resulting from hybridization (Johnson and Fulton 2004, Allen et al. 2009, Lamothe 2013), it has been observed for bass in more northern latitudes (Philipp and Claussen 1995, Philipp et al. 2002) and in laboratory settings (Cooke et al. 2001, Cooke and Philipp 2006, Goldberg et al. 2005).

Summary

While this represents a small sample of northeast Arkansas streams, the presence of FB alleles in bass remote from AGFC stockings should pose concern. Introductions of FB, whether intentional or accidental, into existing native LMB populations may persist for long periods of time. As it is unknown at this time whether fitness is lowered in Arkansas bass as a result of FB alleles, caution should prevail. Stream systems are open and continuous, so that alleles introduced in one area can be moved within these systems. Short-term goals of providing larger bass to anglers must be tempered with the potential of long-term consequences of introducing alleles of unknown impact. Further, we recommend that a larger analysis of bass within Arkansas stream systems be conducted to identify the extent of introductions of FB alleles into those systems.

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Investigating the Effect of Stratospheric Radiation on Seed Germination and Growth

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Running title: Investigating the Effect of Stratospheric Radiation on Seed Germination and Growth

Abstract

Three seed types: bean (*Phaseolus vulgaris*), corn (*Zea mays*) and radish (*Raphanus sativus*) were flown in a high altitude weather balloon into the mid-stratosphere to investigate the effects of high altitude radiation on germination success and seedling growth. After recovering and planting the seeds, the bean seeds showed lower germination success with exposure to high altitude radiation, and consequently stunted seedling growth. Corn and radish seeds experienced a statistically significant positive effect on germination success from radiation exposure compared to control seeds, but negative effect on seedling growth. Overall, the field experiments presented here support laboratory studies that show radiation exposure on vegetable seeds has a mixed effect on the germination success and negative effect on seedling growth on investigated seed types.

Introduction

With the advent of climate change and the variation in the ozone layer thickness, it is expected that more harmful incoming radiation will reach the lower tropospheric regions. The variation in the ozone layer has been influenced by many factors including anthropogenic activities such as the use of chlorofluorocarbons (CFCs), which have a destructive effect on the ozone layer. The amount of radiation, specifically ultraviolet (UV) reaching the surface depends greatly on the strength of the ozone layer (Krupa 2000). Radiation greatly impacts several physiological and biochemical process in animals and plants (Solomon 1999). Higher radiation levels have been shown to lead to skin damage, generalized DNA damage, eyesight loss in humans, inhibition of cress seedlings and limitations on the anthrocyanin

formation in corn (Madronich et al. 2011). Exposure to radiation (specifically, UV) has also been shown to interfere with protein synthesis processes, water exchange, enzyme activity, and leaf-gas exchange (Stoeva and Bineva 2001) in plants. For example, percent germination in seeds and growth rates of seedlings were found to be inversely related to radiation doses in kabuli chickpea plants (Hameed et al. 2008), rice (Maity et al 2005) and in corn and sunflower (Mark and Tevini 1996)

Laboratory research has already investigated the effect of solar irradiation on plants, by exposing the plants to UV in growth chambers (Hollósy 2002). UV-B radiation artificially supplied via filtered lamps into growth chamber was found to impact photosystems I and II, carboxylating enzymes, stomatal resistance, chlorophyll concentrations, soluble leaf proteins, lipids, and carbohydrate pools (Teramura 1983, Hu et al. 2013) While important data have been collected by these studies, these experiments were conducted indoors, creating unrealistic conditions that may exaggerate the influence of radiation on plant processes. This creates a need for field experiments, where the seeds are exposed to radiation in the atmosphere, and their processes investigated to identify what effect the radiation has on plant growth. In this study, we expose three seed types: garden bean (*Phaseolus vulgaris*), corn (*Zea mays*) and radish (*Raphanus sativus*) to stratospheric radiation by using high altitude weather balloons and use germination success and stem growth as indicators to study the effect of radiation on seeds.

Materials and Methods

A 1200 gram latex atmospheric weather balloon was used to lift payload boxes containing high altitude experiments and atmospheric monitoring instruments

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into the lower stratosphere. The balloon was filled with helium and calibrated to ascend 370 m/min (1200 ft/min) up to 26 km (100,000 ft.) with a flight time of around 90-120 minutes. Payload boxes are attached to the balloon through 100 lb. test strength polyester string line that runs through the middle of the payload boxes. At the end of the line, there was an antenna and GPS recorder for balloon communication and tracking. In between the balloon and the antenna were other payload boxes containing atmospheric monitoring instruments (HOBO data collectors and loggers), and the experimental payload box with the seeds for this study. These data collectors and loggers measured properties of the atmosphere (temperature, pressure, water vapor). A radiosonde (model AnaSonde-2G Anasphere Inc.) collected temperature, pressure, and water vapor while Vernier UV sensors (UVA-BTA and UVB-BTA) measured the ultraviolet exposure during balloon flight. Vernier manufactures education grade equipment, so ultraviolet exposure measured is approximate.

The experiment payload box (Fig. 1) was made of a foam-board measuring 10 x 22 x 30cm (3.5 x 9 x 12") and coated with a heavy duty water repellant (Silicone water guard by Sno-Seal, Item#1336) to keep the box dry. Six 24 Multiwell™ tissue culture plates were mounted at each side of the box, three outside and three inside the box and secured with screws. Seeds were then added into the culture plates and the lids closed securely. Three seed types were used: bean (*Phaseolus vulgaris*), corn (*Zea mays*) and radish (*Raphanus sativus*). Each 24 Multiwell™ tissue culture plates was packed with 60-72 vegetable seeds, with 3-5 seeds per well. The seeds were divided into three groups: outside, inside and control. Outside seeds were placed outside the payload box for full exposure to high altitude radiation. Inside seeds were placed inside the payload box protected from the outside radiation. Control seeds were left in the laboratory, as a control group with exposure to normal lower altitude background radiation levels.

The weather balloon and payload boxes were launched into the mid-stratosphere. As the balloon ascends the pressure difference between the ground and mid-stratosphere causes the balloon to expand until, at high enough altitude, the balloon bursts. The payload boxes then descends in free fall until reaching the lower troposphere. Then a parachute attached under the balloon is able to catch air and slow the payload boxes for a safe landing on the ground. GPS transmissions by radio allowed tracking and retrieval of the payloads.

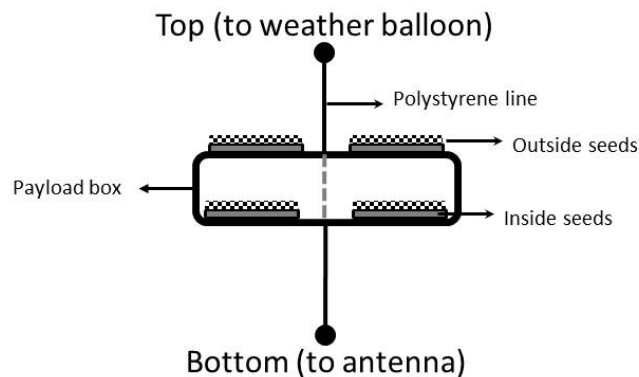


Fig. 1: Experimental payload apparatus showing the different arrangements of seeds in/out of the payload box. The seeds are secured inside multiwell plates, three plates on top, and three inside (only two shown here). Each multiwell plate was covered (checkerboard pattern) with a clear plastic lid.

Under high altitude conditions, outside seeds (outside the payload box) came into contact with low higher levels of radiation (beta and gamma) compared to inside seeds lower levels of radiation because the box shielded gamma and beta radiation. The condition each seed type was exposed to is shown in Table 1.

Table 1: Experimental conditions experienced by the seeds during flight and in the control systems.

Conditions	Seed placement	
	Inside the payload	Outside the payload
Flight time (min.)	90-120	90-120
Max altitude (km)	26	26
Temperature (K) (min-max)	214-326	214-326
Pressure (KPa) (min-max)	0.960-95.0	0.960-95.0
Water vapor content (gm/m ³) (min-max)	4.1-12.4	4.1-12.4
UVA exposure (mW/m ²) (min-max)	0	60-3053*
UVB exposure (mW/m ²) (min-max)	0	14-108*

*approximate exposure as collected with the Vernier, which is an education grade instrumentation

Seeds were removed from the Multiwell plates and planted under 0.25-0.5 inch of soil two days after exposure to high altitude conditions. Varying amount of seeds were planted due to how many seeds were

packed into each “well”; 60 corn seeds and 72 bean, and 72 radish seeds were used. Holes were punched into the bottom of germination trays to allow for excess water to drain out of tray. Seeds were placed in a greenhouse and watered every day. Germination success and seedling growth were measured 7 days after planting seeds. Percent germination success was identified by how many seeds germinated from the total seeds planted, while seedling growth was analyzed by measuring the stem length from the soil to the top of the stem.

Statistical analyses were performed in Excel and Kaleidagraph. Briefly, the horizontal bars in the box represent the group median, the box boundaries represent the 25th and 75th percentile values (LQ and UQ respectively) and the “whiskers” in either end represent maximum and minimum values within 1.5 the interquartile distance (IQD), the distance between the upper and lower quartiles (UQ-LQ). Values outside this range are defined by two equations (eq.1-2) where outliers and marked as individual points.

$$\text{height} > UQ + 1.5 \times IQD \quad \text{eq. 1}$$

$$\text{height} < LQ - 1.5 \times IQD \quad \text{eq. 2}$$

Results and Discussion

The effect of radiation on seeds was followed by investigating the percent of seeds that germinated. Germination success rates for each type of seeds are shown in Fig. 2. Beans seeds that were in the laboratory (as control) have a germination success of around 88.9%. The seeds that were sheltered (inside the payload box) had a germination success rate of 93.1%. The seeds that were outside the box, exposed to high radiation showed a germination success of 83.3%.

The germination success for control corn seeds (in the laboratory) was measured at 83.3%. The corn seeds that were inside the box (inside) had a success percentage of 63.8%, a lower success than the control seeds, while the seeds exposed to radiation (outside) have an 86.1%, a slightly higher germination success rate than the control. Radish was also found to increase in germination success upon exposure to radiation. The radish seeds that were outside the payload box showed a 86.1% germination success rate, compared with 77.8% for the control seeds and 76.4% for the seeds inside the payload box. The comparison of germination rates are summarized in Fig. 2.

The total height of control bean plants were found to be statistically different than outside bean plants ($p=5.296E-5$) and inside bean plants ($p=1.768E-3$).

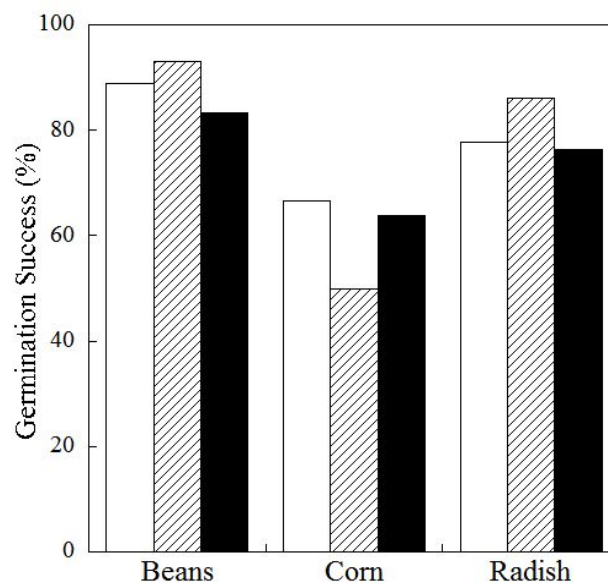


Fig. 2: Germination success with 5% error measured as a percentage of seeds germinated verses seeds planted of a certain type and exposure. The seeds were grouped as control (clear), inside (diagonal), and outside (dark).

Inside bean plants are not statistically different in height compared to outside bean plants ($p=2.844E-1$).

Bean seedlings have been shown to be sensitive to UVB irradiation and water vapor content. It has been observed that exposure to UVB radiation can halve the fresh weight and leaf area of bean seedlings in comparison to control seedlings that had no UVB irradiation (Tevini, et al. 1981). Rapid desiccation of bean seeds was found to harm seed integrity and impact seedling vitality (Sanhewe and Ellis 1996).

Control radish plant height is not statistically different than outside radish plant height ($p=4.688E-1$), but is statistically different than inside radish plant height ($p=3.956E-2$). Inside radish plants are statistically different in height compared to outside radish plants ($p=1.721E-5$). Control corn plant height is not statistically different than outside corn plant height ($p=8.345E-1$), but is statistically different than inside corn plant height ($p=9.351E-2$). Outside corn plant height is not statistically different than inside corn plant height ($p=1.068E-1$). All p-value calculations are summarized in Table 2.

Seed germination in outside bean seeds (exposed to radiation) was not significantly affected in comparison to the control, with a decrease of around 5.6%. The inside bean seeds showed no effect from the radiation. This indicates exposure to radiation does not significantly affect the germination success of bean seeds.

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Table 2: P values comparing seedling height of different group of seeds. P <0.05 indicated with a star show compared groups are statistically different.

Group 1	Group 2	p
Control bean	Outside bean	5.296E-5 *
Control bean	Inside bean	1.768E-3 *
Inside bean	Outside bean	2.844E-1
Control corn	Outside corn	8.345E-1
Control corn	Inside corn	9.531E-2 *
Inside corn	Outside corn	1.068E-1
Control radish	Outside radish	4.688E-1
Control radish	Inside radish	3.956E-6 *
Inside radish	Outside radish	1.721E-5 *

The germination success of outside corn seeds show slightly higher germination success compared to control. This increase can be explained by UV promotion. Epigenetic research has shown that UVC radiation affected germination rates due to shifts in methylation patterns of satellite and transcribed DNA (Sokolova et al. 2014). The very low germination success rate of corn inside the box cannot be explained, presently. Outside radish seeds had a higher percent germination compared to control, possibly due to UVA growth promotion. This promotion was previously found to be associated with an increase in chlorophyll content and photosynthetic activity (Tezuka et al. 1994).

When the seedling growth (as measured by stem height from the soil) was investigated, there were no significant changes between the three seed types investigated as seen in Fig 3b. The inside bean seeds at 17 cm show a faster growth rate than the control (13 cm), close to the average height of outside bean seeds which was measured at 18 cm (about 38% higher than the controls). The average corn plant height for the outside seeds were measured at 7 cm, slightly less than the control seeds (8 cm), while the inside seeds height were significantly lower (6.2 cm), than the controls seeds as seen in Fig. 3b. The improvement on growth rates of seedling height with radiation exposure has also been observed with rice (*Oryza sativa* L.) although, the growth rates were found to decrease upon further radiation exposure (Maity et al. 2005).

Inside radish seeds were statistically significantly shorter than control radish seeds and outside radish seeds (Table 2). The average height of radish plants (2.5 cm) was shorter than the average height of outside and control seeds (3 cm) as seen in Fig 3c. The distribution of the plant height was not affected

because the size of the box, which represents 85% of the data set that fits within 95%, is the same height (about 1 cm difference). The outside radish seeds are taller than inside radish seeds because outside seeds are exposed to higher levels UV radiation in addition to cold temperatures. Growth promotion has been shown with UVA exposure to radish seeds, and has been explained by an increase in chlorophyll content and photosynthetic activity from the ability to undergo cellular respiration (Tezuka, et al. 1993). Radish seedlings were not found to be influenced by UVB irradiation (Tevini, et al. 1981). Therefore, the increase in germination success must be influenced by UVA exposure and not UVB exposure.

Plans for future research include measuring UVC profile to quantify the exposure on the seeds. Also, plans to look into the F1 and F2 generations of plants, specifically seedling height and fecundity.

Conclusions

Bean, corn and radish seeds were transported to the mid stratosphere in a payload box carried by a weather balloon and exposed to stratospheric radiation. The seeds were planted in soil to investigate the effect of radiation on their germination and growth. All seeds types responded to exposure to radiation, with the bean seeds showing a negative effect on germination success but a slight enhancement in seedling growth compared to control. The corn and radish seeds exposed to radiation had a higher germination success when compared to control, but a mixed response compared to control. The field experiments presented here support other laboratory studies on germination and seedling growth, highlighting another important aspect on the response of vegetation to the change in radiation levels due loss of ozone layer resulting from climate change.

Acknowledgements

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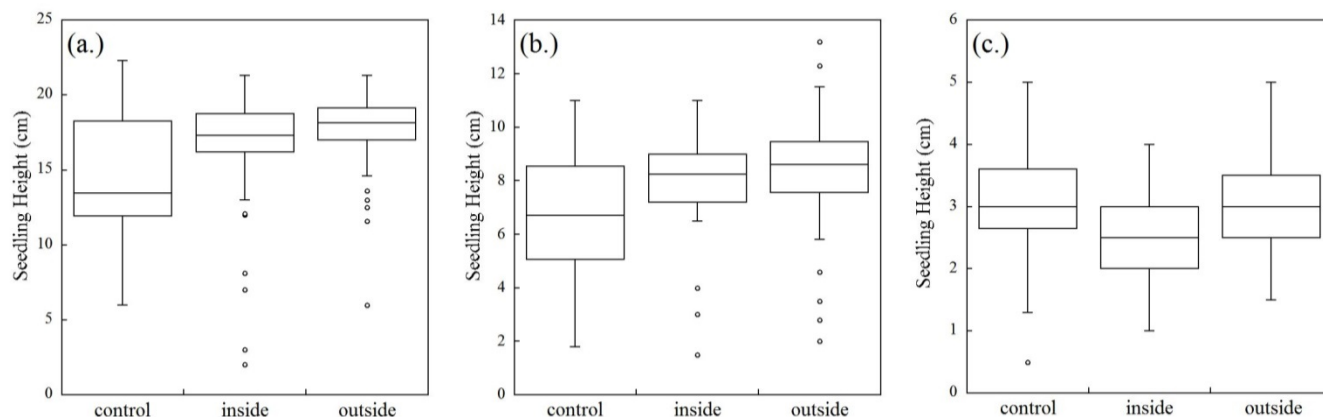


Fig. 3: Box plot of seedling growth of different seeds on a) beans, b) corn, and c) radish. Points represent outliers that are outside the range 1.5x interquartile distance.

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Development of Heterogeneous Photosensitized Transition Metal Oxide Water-Splitting Catalysts on Silica Support

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Running Title: Development of Photosensitized Water Splitting Catalysts

Abstract

The research presented in this manuscript describes the development of photosensitized inexpensive catalysts based on readily available materials. The investigation covers synthesis and characterization of photosensitizers based on porphyrins, mechanical and thermal coating of solid support with semiconducting transition metal oxides, photosensitization of the semiconducting layer, and characterization of the photoelectrochemical properties displayed by the new materials. The process of water oxidation is of primary interest here, with little emphasis put on reduction of protons to gaseous hydrogen. Photoelectrochemically produced protons serve as a probe of effectiveness of the catalysts. Several systems are described, and two catalysts are identified as the most efficient.

Introduction

The photoinduced process of water splitting is currently under heavy investigation by several research groups (O'Regan and Grätzel 1991). There are a number of reasons why there is so much interest in water splitting. For example, because the primary source of energy harvested to power the process (effectively converting it to a technologically useful type) is sunlight, the process is "green" in every aspect; the process is also nearly perfectly environmentally friendly due to the renewability of the materials, carbon emission reduction or, complete elimination and the multiple uses for the products of this seemingly simple reaction, namely: oxygen and especially hydrogen. Since technologies already exist that allow for production of renewable and extremely clean energy from hydrogen, e.g. hydrogen cells, cheap mass production of this excellent fuel is highly desirable (Yilanci et al. 2009). Even though several catalysts were developed to facilitate this process, there are multiple factors that render them unsuitable for scale-up; for example cost, low efficiency, rare materials

used for their production, very high purity required, technological challenges, etc. (Bloor et al. 2014). To target a few of these challenges, the following approach is presented in this paper: the components used are inexpensive and readily available; the process of making the catalysts is simple, quick and requires equipment of minimal complexity. The overall goal was to produce a heterogeneous, granular catalyst based on silica coated with a semiconducting photosensitized transition metal oxide.

Figure 1 schematically represents the underlying mechanisms involved in photocatalyzed water-splitting, occurring on and in a granule of the catalyst. Process "A" in Figure 1 involves: a) absorption of photon(s) by the outside layer, which is the photosensitized part of the semiconductor, b) injection of excited, high energy electrons into the conducting band of the semiconductor from its valence band, facilitated by the photosensitizer, and c) production of electron holes in the semiconductor. This mechanism is coupled with process "B": a) contact between water molecules and the semiconductor surface, b) transfer of electrons from water molecules to electron holes, resulting in water oxidation, c) production of gaseous

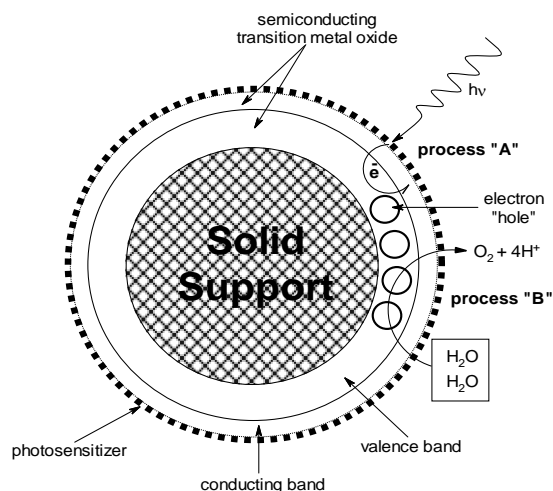


Figure 1. Model of the water splitting mechanisms.

diatomic oxygen and d) production of protons. The increasing proton, or appropriately, hydronium ion concentration quantified over time correlates with efficiency of the system.

The heterogeneous nature of such catalysts offers very high flexibility in coupling of this process and the subsequent (but physically separate) proton reduction, if desired, to gaseous diatomic hydrogen. After a specified low-limit pH is obtained in the light harvesting aqueous suspension, the acidic solution can be filtered and pumped to a complimentary reducing environment in an automated and cyclic mode, returning the catalyst to a fresh batch of neutral water. However, this secondary process is beyond the scope of the current report.

Materials and Methods

The semiconducting metal oxides were purchased from the following suppliers: zirconium dioxide (Alfa Aesar, 99.7%); zinc oxide (Alfa Aesar, 99.999%); titanium dioxide (Loud Wolf LTD, 99.9%); tungsten oxide (Alfa Aesar, 99.998%). Silica 60 (70-230 mesh), pyrrole (98%), propionic acid (99%), zinc acetate dihydrate (97%) were all acquired from Alfa Aesar. Florisil (60-100 mesh) was purchased from J.T. Baker, solvents (reagent grade and better) from Fisher and Macron; *meso*-5,10,15,20-tetrakis-2-carboxyphenyl porphyrin (*o*-TCPP) and *meso*-5,10,15,20-tetrakis-3-carboxyphenyl porphyrin (*m*-TCPP) standards were purchased from Frontier Scientific, and *ortho*-carboxybenzaldehyde and *meta*-carboxybenzaldehyde from ACROS (99%). The lamp used for irradiations was a Bayco 500 W halogen. Measurements were performed with Vernier temperature and pH probes (Logger Pro software) and a Bruker FT-IR ALPHA (Platinum ATR) instrument.

Organic synthesis of photosensitizing dyes was performed according to known methods (Adler et al. 1964, Rothmund 1935, 1936), based on condensation of pyrrole and substituted benzaldehydes in propionic acid as a solvent. Small, pure samples of compounds were acquired as references, from commercial sources.

meso-5,10,15,20-tetrakis-2-carboxyphenyl-porphyrin: pyrrole (1.688 g, 25.16 mmol) and 2-carboxybenzaldehyde (3.808 g, 17.53 mmol) were mixed with propionic acid (190 mL). The mixture was refluxed for 30 minutes, cooled to room temperature, and then placed in an ice bath for 10 minutes. Filtration and purification (silica column, AcOEt/Hexane/MeOH 75:24:1) yielded the desired free base porphyrins. Figure 2 below schematically shows the method.

Metallation (Zn) of the photosensitizers was performed in order to improve their efficiency according to known methods of metal cation insertion into the porphine core (Kadish et al. 1999); tetraphenylporphyrin, TPP, (300 mg, 0.488 mmol) in CHCl_3 (20 mL) were added to a solution of $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ (123 mg, 0.560 mmol) and MeOH (3 mL). The reaction reached completion after 3 hours. Completion of the reaction was determined by TLC analysis using a dichloromethane/acetone/acetic acid (8:2:0.1) solvent system. Formation of a new more polar green band signaled completion. This procedure was repeated using pure *ortho*-TCPP (30 mg, 38 μmol) and pure *meta*-TCPP (30 mg, 38 μmol) using a dichloromethane/ CHCl_3 (1:1) solvent system. All reactions proceeded in ~quantitative yield as described before (Wang et al. 2005); see Figure 2.

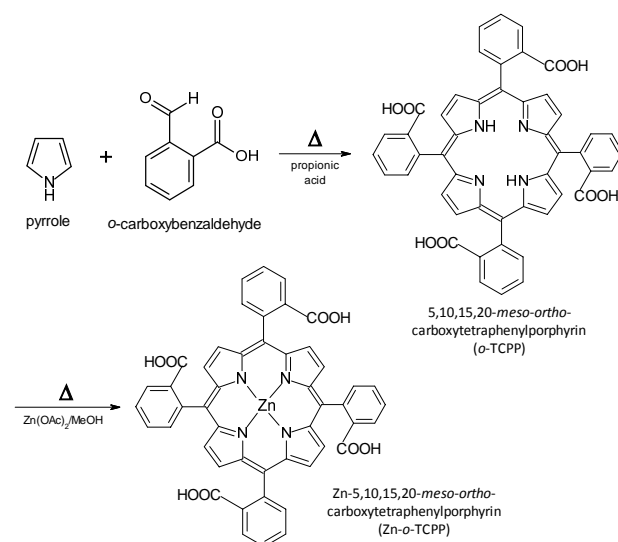


Figure 2. General method of porphyrin synthesis.

Coating and thermal fusion of silica support with semiconducting transition metal oxides was done by vigorous mechanical agitation of silica samples (10 g) with metal oxides (5 g) for 10 minutes, followed by calcination in a furnace for three hours; the oxides used were TiO_2 (450°C), ZnO (350 °C), WO_3 (400°C), ZrO_2 (400°C).

TPP (12 mg, 20 μmol) was added to dichloromethane (20 mL) to make a 0.98×10^{-4} M solution. The TPP solution was added to a 100 mg sample of the calcined SiO_2 - TiO_2 mixture, with minimal stirring for 60 minutes. The heterogeneous mixture was filtered and dried for 10 minutes. This procedure was repeated for samples of SiO_2 -ZnO, SiO_2 - WO_3 , and SiO_2 - ZrO_2 .

Development of Photosensitized Water Splitting Catalysts

Zn-TPP (14 mg, 21 μmol) was added to dichloromethane (20 mL) to make a 1.0×10^{-3} M solution. The Zn-TPP solution was added to a 100 mg sample of the calcined $\text{SiO}_2\text{-TiO}_2$ mixture, with minimal stirring for 60 minutes. The solution was filtered and dried for 10 minutes. This procedure was repeated for samples of $\text{SiO}_2\text{-ZnO}$, $\text{SiO}_2\text{-WO}_3$, and $\text{SiO}_2\text{-ZrO}_2$.

Zn-*ortho*-TCPP (16 mg, 19 μmol) was added to dichloromethane (20 mL) to make a 9.3×10^{-4} M solution. The Zn-*ortho*-TCPP solution was added to a 100 mg sample of the calcined $\text{SiO}_2\text{-TiO}_2$ mixture, with minimal stirring for 60 minutes. The solution was filtered and dried for 10 minutes. This procedure was repeated for samples of $\text{SiO}_2\text{-ZnO}$, $\text{SiO}_2\text{-WO}_3$, and $\text{SiO}_2\text{-ZrO}_2$.

Zn-*meta*-TCPP (16 mg, 19 μmol) was added to dichloromethane (20 mL) to make a 9.3×10^{-4} M solution. The Zn-*meta*-TCPP solution was added to a 100 mg sample of the calcined $\text{SiO}_2\text{-TiO}_2$ mixture, with minimal stirring for 60 minutes. The solution was filtered and dried for 10 minutes. This procedure was repeated for samples of $\text{SiO}_2\text{-ZnO}$, $\text{SiO}_2\text{-WO}_3$, and $\text{SiO}_2\text{-ZrO}_2$.

After drying, all samples were then heated on a hot plate at 80 $^\circ\text{C}$ for 10 minutes to allow for complete evaporation of the solvent and adsorption of the porphyrins with the transition metal oxide surface on the silica support.

Measurements

All irradiation trials consisted of a 35 mg sample of the catalysts in 10 mL of deionized water. The solution was placed in a 20 mL beaker with a spin vane. The 500 W halogen lamp was placed 50 cm away from the beaker (non-concentrated light). Over the course of 60 min the pH and temperature of the solution were recorded. Between each trial, the pH probe, temperature probe (thermocouple), beaker and spin vane were thoroughly cleaned.

The temperature monitored during the experiments was not allowed to exceed 42 $^\circ\text{C}$. It was determined experimentally that 50 cm distance between the samples and the lamp was needed to avoid errors in interpretation of oxygen evolution due to the thermally decreased solubility of the gas in water.

The irradiation *controls* consisted of SiO_2 , ZnO, TiO_2 , WO_3 , ZrO_2 , $\text{SiO}_2\text{-ZnO}$, $\text{SiO}_2\text{-TiO}_2$, $\text{SiO}_2\text{-WO}_3$, $\text{SiO}_2\text{-ZrO}_2$, TPP, ZnTPP, Zn-*ortho*-TCPP, and Zn-*meta*-TCPP.

The irradiation *trials* consisted of $\text{SiO}_2\text{-TiO}_2\text{-TPP}$, $\text{SiO}_2\text{-TiO}_2\text{-Zn-TPP}$, $\text{SiO}_2\text{-TiO}_2\text{-Zn-ortho-TCPP}$, $\text{SiO}_2\text{-TiO}_2\text{-Zn-meta-TCPP}$.

$\text{TiO}_2\text{-Zn-meta-TCPP}$, $\text{SiO}_2\text{-ZnO-TPP}$, $\text{SiO}_2\text{-ZnO-Zn-TPP}$, $\text{SiO}_2\text{-ZnO-Zn-ortho-TCPP}$, $\text{SiO}_2\text{-ZnO-Zn-meta-TCPP}$, $\text{SiO}_2\text{-WO}_3\text{-TPP}$, $\text{SiO}_2\text{-WO}_3\text{-Zn-TPP}$, $\text{SiO}_2\text{-WO}_3\text{-Zn-ortho-TCPP}$, $\text{SiO}_2\text{-WO}_3\text{-Zn-meta-TCPP}$, $\text{SiO}_2\text{-ZrO}_2\text{-TPP}$, $\text{SiO}_2\text{-ZrO}_2\text{-Zn-TPP}$, $\text{SiO}_2\text{-ZrO}_2\text{-Zn-ortho-TCPP}$, $\text{SiO}_2\text{-ZrO}_2\text{-Zn-meta-TCPP}$.

Please, note that the study did not include proton reduction catalysts in order to make monitoring of the pH drop (increase of the H^+ concentration) possible.

Results and Discussion

The designed photocatalysts performed as expected; a consistent decrease of the pH was observed during the irradiation experiments, accompanied by evolution of oxygen bubbles. TPP was eliminated as a suitable photosensitizer due to the lack of adherence to catalysts' surface. Both: *ortho*-carboxy and *meta*-carboxytetraphenyl porphyrins displayed strong enough binding to the transition metal oxides to efficiently perform their designed function in a stable manner.

Figure 3 shows the representative pH decrease caused by photoirradiation of suspensions of the two most efficient photosensitized catalysts, namely $\text{SiO}_2\text{-TiO}_2$ photosensitized with Zn-*ortho*-TCPP and $\text{SiO}_2\text{-ZrO}_2$ photosensitized with Zn-*meta*-TCPP.

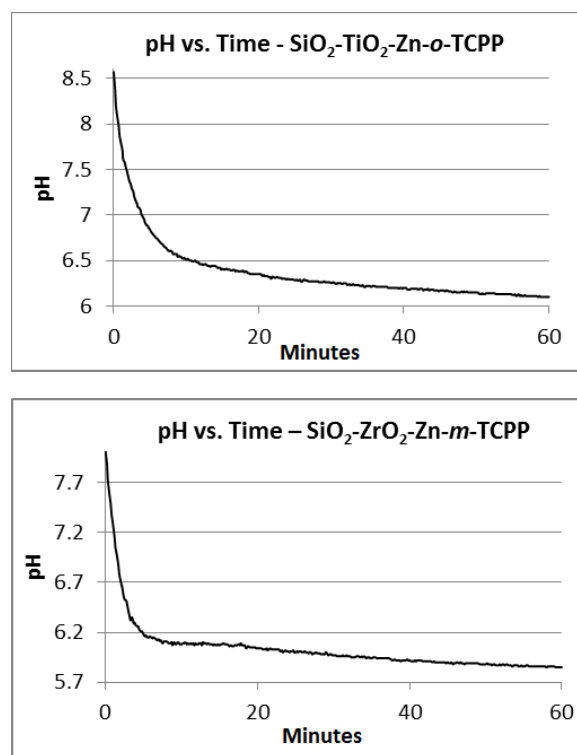


Figure 3. Representative pH decrease caused by photoirradiation facilitated by the two most efficient catalysts.

Table 1. Total change of pH after 60 min of photoirradiation of the tested photosensitizers.

	Photosensitizers					
	Zn-o-TCPP			Zn-m-TCPP		
Catalyst	Initial pH	Final pH	Δ pH	Initial pH	Final pH	Δ pH
SiO ₂ -TiO ₂	8.57	6.1	-2.47	6.69	5.55	-1.14
SiO ₂ -WO ₃	5.94	5.57	-0.37	7.87	7.07	-0.8
SiO ₂ -ZrO ₂	7	5.51	-1.49	8.02	5.85	-2.17
SiO ₂ -ZnO	6.15	6.98	0.83	8.58	7.84	-0.84

A summary of all results is included in Table 1. All control data are available upon request.

Oxygen gas evolution was documented by photographing the irradiated suspensions of the catalysts in DI water. Figure 4 shows the gas bubbles emerging during the process. Quantitative analysis of the gas produced was beyond the scope of this research.



Figure 4. Oxygen bubbles emerging from the photoirradiated heterogeneous mixture: water and SiO₂-TiO₂-Zn-o-TCPP.

The least active catalyst was based on tungsten oxide. Catalysts based on zinc oxide and sensitized with Zn-*ortho*-TCPP noticeably *increased* the pH of the solution, presumably due to the formation of zinc hydroxide which dissociates to a very small extent and increases the concentration of hydroxide ions in aqueous solutions.

Conclusions

The most active photocatalysts from the series have been identified and shown to be potential

photocatalytic water-splitting catalysts. The most efficient combinations were titanium oxide sensitized with Zn-*ortho*-TCPP and zirconium oxide sensitized with Zn-*meta*-TCPP. The least active catalyst was based on tungsten oxide; zinc oxide proved to be unsuitable for this application. Future goals include design and investigation of more efficient organic photosensitizers.

Acknowledgements

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Bond Length – Bond Valence Relationships for Carbon – Carbon and Carbon – Oxygen Bonds

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Running Title: Bond Length – Bond Valence Relationships for C-C and C-O Bonds

Abstract

In the present study, relationships are developed for determining bond orders (also referred to as bond valences or bond numbers) from published bond lengths for carbon-carbon (C-C) and carbon-oxygen (C-O) bonds. The relationships are based on Pauling's empirical formula $s = \exp((R_o - R)/b)$, where s is the bond order, R is the corresponding bond length, R_o is the unit valence bond length, and b is a fitting parameter. We use a recently derived relationship for the b parameter in terms of the bonding atoms' published atomic orbital exponents. The resulting equations were checked against published x-ray diffraction (XRD) data for 176 carbon systems with 540 published C-C bond lengths, and 50 oxygen systems having 72 published C-O bond lengths. The C-C and C-O bond length-valence relationships are shown to have sufficient applicability and accuracy for use in any bonding environment, regardless of physical state or oxidation number.

Introduction

In 1929, Linus Pauling published his five rules of chemical bonding which could be used for predicting crystal structures (Pauling 1929). Pauling's second rule is that of local charge neutrality, commonly known as the valence sum rule, which states that the charge of an anion is neutralized by the sum of the adjacent cationic charges, while any cationic charge is neutralized by adjacent anionic charges. In terms of bond valence, the total valence at any one atom is equal to the sum of that atom's individual bond valences. In 1947, Pauling published the following empirical bond length-valence relationship:

$$s = \exp\left(\frac{R_o - R}{b}\right) \quad (1)$$

where s is the bond valence, which corresponds to the number of lone pairs of electrons contributing to the bond, R_o is the length of a chemical bond with unit valence, R is an observed bond length, and b is an empirical fitting parameter (Pauling 1947). A wide range of determined values for the b parameter, anywhere from 0.25 to 0.65 Å (Hardcastle 2013), led to many inconsistencies in valence values, an issue that hindered the ability of chemists to compare findings. As a result, it was proposed that a universal value of 0.37 Å for b be established as the average from the crystallographic data (Brown and Altermatt 1985). This resulted in consistent relationships having only one fitting parameter, R_o ; however, when applied to shorter and longer bonds, the calculated valence was shown to be less reliable owing to the inaccuracy of b .

Theory

In 2013, Hardcastle derived Pauling's bond length-valence, including a new definition for the b fitting parameter (Hardcastle 2013). Since then, a slight modification has been made (Hardcastle *unpublished data*), resulting in the following equation:

$$b = \frac{2a_o}{(\xi_1 + \xi_2)} \quad (2)$$

where b is dependent upon the Bohr radius of a hydrogen atom, a_o (0.529 Å), and the sum of the orbital exponent values for each of the atoms contributing to the bond. This definition results in values for b that are specific to the type of chemical bond, a much more accurate alternative to the average universal value of 0.37 Å assumed for any type of bond. Using published values for atomic orbital exponents to determine the value of the b parameter for any bond, and substituting this value into Equation (1), results in a bond length-valence relationship specific to that bond type.

Uncertainty in R_o

With a definition for the b parameter, R_o is left as the only fitting parameter in Equation (1). The precise length of a C-O bond having a bond order of exactly one (a true C-O single bond) is a matter of debate, but has been estimated between 1.33 and 1.43 Å by Allen (Allen et al 1987) and at 1.39 Å by Brese and O'Keeffe, which they refer to as the "bond-valence parameter" because they were using $b = 0.37$ Å as a universal constant (Brese and O'Keeffe 1991). For the C-C bond length of unit valence, however, most investigators agree on the published C-C length found for crystalline diamond at $R_o = 1.542$ Å (Brown 2002) as representing the C-C bond length of unit valence.

Results and Discussion

The atomic orbital exponents for carbon and oxygen are from data published by Clementi and Raimondi (1963), with values of 1.5679 and 2.2266 respectively. Substituting these values into Equation (2), results in b parameters of 0.337 Å for C-C bonds and 0.279 Å for C-O bonds. Note that both of these values are much lower than the previously assumed universal constant of 0.37 Å. This leaves R_o , the bond length of unit valence, as the only remaining fitting parameter. In the case of C-C bonds, our initial guess would be the C-C bond length in diamond at $R_o = 1.542$ Å. But for C-O bonds, this value could be anywhere from 1.33 (Allen et al 1987) to 1.43 Å (Schomaker and Stevenson 1941).

The total atom valence for a carbon-centered environment was predicted to be 4.00, carbon having four electrons available for bonding, while the atom valence for an oxygen-centered environment was predicted to be 2.00. These predictions were based upon the number of bonding electrons available (oxidation state) in each atom, 4 for carbon and 2 for oxygen. Comparing the calculated atomic valences to the predicted valences, the total error for the C-O bonding was minimized by manipulating R_o . Clementi's orbital exponents were not changed to minimize error, but were held constant.

X-ray diffraction data, limited to results published in the year 2000 or later, was collected for C-C and C-O bond lengths, totaling 612 bonds (176 carbon environments and 50 oxygen environments). Each environment is represented by an individual table within Table 1. Bond lengths were recorded and converted to bond valence values, which were then totaled for the atom valence. Data analysis and error

minimization led to two specific relationships, one for C-C bonds:

$$s = \exp\left(\frac{1.5420 - R}{0.337}\right) \quad (3)$$

and one for C-O bonds:

$$s = \exp\left(\frac{1.3669 - R}{0.279}\right) \quad (4)$$

Each equation was shown to produce accurate valence values from published bond length data. The error in the data (XRD data and valence sum rule) was minimized at $R_o = 1.5420$ Å for C-C bonds, consistent with the C-C bond length of diamond, and $R_o = 1.3669$ Å for C-O bonds, consistent with the estimated 1.33-1.44 Å range for a C-O bond of unit valence.

Conclusion

Bond length – bond valence relationships, based on Pauling's formula, provide useful tools for the prediction and evaluation of crystal structures when used with the valence sum rule. In the present study, atomic orbital exponents were used to independently determine the value of the b parameter (previously either a floating fitting parameter, or set as a universal constant at 0.37 Å) for C-C and C-O bonds at 0.337 and 0.279 Å, respectively. This approach resulted in bond length – valence relationships for C-C and C-O bonds by using published crystallographically determined bond lengths for 612 bonds and the valence sum rule. The optimized bond lengths of unit valence are minimized at $R_o = 1.5420$ Å for C-C bonds, consistent with the C-C bond length of diamond, and $R_o = 1.3669$ Å for C-O bonds, consistent with the estimated 1.33-1.44 Å range for a C-O bond of unit valence.

Acknowledgments

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Bond Length – Bond Valence Relationships for C-C and C-O Bonds

Table 1. Bond valence calculations from published XRD data.

<i>Carbon Environments</i>														
Alonso et al. 2009			C13	1.338	1.831	C9	Valence		C36	1.403	1.510	C2	Valence	
			C17	1.508	1.106	C4	1.429	1.398	C43	1.517	1.077	C1	1.423	1.423
			O1	1.360	1.025	C8	1.390	1.569			4.035	C3	1.204	2.723
					3.962	C10	1.525	1.052			C39	Valence		4.146
C10	1.474	1.223		C16	Valence			4.019	C34	1.398	1.532		C3	Valence
O2	1.210	1.755	C15	1.341	1.814		C40	Valence	C38	1.398	1.532	C2	1.204	2.723
O3	1.304	1.253	C21	1.504	1.119	C35	1.410	1.479	C40	1.520	1.067	C4	1.431	1.390
			O1	1.361	1.021	C39	1.397	1.537			4.132			4.113
					3.955	C41	1.517	1.077	Borbulevych et al. 2002				C6	Valence
				C12	Valence			4.093		C8	Valence	C5	1.423	1.423
C10	1.475	1.220	C1	1.393	1.555		C36	Valence	C7	1.500	1.133	C7	1.200	2.756
O2	1.213	1.737	C13	1.425	1.414	C35	1.413	1.466	C9	1.540	1.006			4.179
O3	1.349	1.066	C15	1.427	1.406	C37	1.395	1.546	O5	1.432	0.792		C7	Valence
					4.376	C44	1.522	1.061	O6	1.430	0.797	C6	1.200	2.756
				C3	Valence			4.073			3.728	C8	1.423	1.423
C10	1.460	1.275	C2	1.381	1.612		C4	Valence		C4	Valence			4.179
O2	1.225	1.663	C4	1.442	1.345	C3	1.426	1.410	C3	1.410	1.479		C9	Valence
O3	1.331	1.137	C5	1.430	1.394	C5	1.390	1.569	C5	1.462	1.268	C8	1.423	1.423
					4.350	C9	1.518	1.074	C9	1.420	1.436	C10	1.205	2.715
				C6	Valence			4.053			4.182			4.138
C10	1.468	1.245	C5	1.474	1.223		C8	Valence	Burlakov et al. 2015				C10	Valence
O1	1.230	1.634	C7	1.387	1.583	C3	1.416	1.453		C7	Valence	C9	1.205	2.715
O2	1.318	1.192	C11	1.383	1.602	C7	1.394	1.551	C6	1.430	1.394	C11	1.430	1.394
					4.408	C12	1.519	1.071	C8	1.203	2.731			4.109
				C26	Valence			4.074			4.125		C13	Valence
			C25	1.502	1.126		C21	Valence		C2	Valence	C12	1.425	1.414
			C27	1.540	1.006	C20	1.408	1.488	C1	1.205	2.715	C14	1.199	2.764
			C28	1.526	1.049	C22	1.400	1.523	C3	1.424	1.419			4.178
			C29	1.531	1.033	C26	1.520	1.067			4.134		C14	Valence
					4.214			4.078	Chen et al. 2009			C13	1.199	2.764
				C20	Valence		C25	Valence		C2	Valence	C1	1.419	1.440
C7	1.483	1.191	C19	1.515	1.083	C20	1.406	1.496	C1	1.431	1.390			4.204
C9	1.514	1.087	C21	1.490	1.167	C24	1.392	1.560	C3	1.190	2.839	Chiang et al. 2001		
C10	1.336	1.841	C22	1.529	1.039	C29	1.520	1.067			4.228		C1	Valence
			C23	1.513	1.090			4.124		C3	Valence	C1a	1.568	0.926
					4.379		C35	Valence	C2	1.190	2.839	O4	1.254	1.499
				C25	Valence	C34	1.408	1.488	C4	1.440	1.353	O3	1.251	1.515
			C24	1.338	1.831	C36	1.394	1.551			4.192			3.940
			C26	1.502	1.126	C40	1.517	1.077		C6	Valence	DiPasquale et al. 2006		
			O1	1.365	1.007			4.115	C5	1.430	1.394		C11	Valence
					3.963		C39	Valence	C7	1.201	2.747	C12	1.522	1.061
				C19	Valence	C34	1.408	1.488			4.141	C13	1.520	1.067
			C18	1.338	1.831	C38	1.396	1.541		C7	Valence	C14	1.525	1.052
			C20	1.515	1.083	C43	1.518	1.074	C6	1.201	2.747	O2	1.458	0.721
			O1	1.357	1.036			4.103	C8	1.436	1.369			3.901
					3.950		C4	Valence			4.117	Draskovic et al. 2010		
				C17	Valence	C3	1.419	1.440		C9	Valence		C2	Valence
			C1	1.391	1.564	C5	1.397	1.537	C8	1.430	1.394	C3	1.436	1.369
			C18	1.420	1.436	C9	1.517	1.077	C10	1.190	2.839	C7	1.380	1.616
			C24	1.424	1.419			4.054			4.232	O1	1.364	1.010
					4.419		C8	Valence		C10	Valence			3.996
				C8	Valence	C3	1.422	1.427	C9	1.190	2.839		C3	Valence
			C7	1.498	1.139	C7	1.392	1.560	C11	1.440	1.353	C2	1.436	1.369
			C9	1.374	1.645	C12	1.512	1.093			4.192	C4	1.429	1.398
			C13	1.377	1.631			4.080		C13	Valence	O2	1.287	1.332
					4.415		C21	Valence	C12	1.430	1.394			4.099
			Basuli et al. 2003			C20	1.419	1.440	C14	1.201	2.747		C4	Valence
				C5	Valence	C22	1.398	1.532			4.141	C3	1.429	1.398
			C4	1.421	1.431	C29	1.520	1.067		C14	Valence	C5	1.419	1.440
			C6	1.391	1.564			4.040	C13	1.201	2.747	C8	1.419	1.440
			C13	1.518	1.074		C35	Valence	C1	1.436	1.369			4.278
					4.070	C34	1.417	1.448			4.117		C10	Valence

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C9	1.537	1.015	C3	1.190	2.839	C	Valence	Liu et al. 2013			4.409			
O3	1.246	1.543	C5	1.430	1.394	C1	1.376	C10	Valence	C9	Valence			
O4	1.265	1.441			4.232	C	1.386	C4	1.398	1.532	C8	1.378	1.626	
3.999				C9	Valence	O	1.378	C5	1.419	1.440	C10	1.401	1.519	
Gavenonis and Tilley			C8	1.420	1.436		4.184	C9	1.415	1.457	O1	1.377	0.964	
2004			C10	1.140	3.292		Valence			4.429			4.109	
	C2	Valence			4.728	C4	1.374		C9	Valence		C2	Valence	
C1	1.412	1.470		C3	Valence	C	1.386	C8	1.371	1.660	C3	1.452	1.306	
C3	1.397	1.537	C2	1.192	2.822	O	1.379	C10	1.415	1.457	O1	1.380	0.954	
C5	1.518	1.074	C4	1.475	1.220		4.191	O1	1.386	0.934	O2	1.203	1.800	
		4.081			4.041		Valence			4.051			4.060	
	C9	Valence		C11	Valence	C6	1.376		C2	Valence	Liu et al. 2014			
C8	1.428	1.402	C10	1.186	2.872	C	1.388	C3	1.437	1.365		C17	Valence	
C10	1.405	1.501	C12	1.459	1.279	O	1.377	O1	1.373	0.978	O2	1.198	1.833	
C14	1.502	1.126			4.151		4.178	O2	1.237	1.593	O3	1.304	1.253	
		4.029	Krawczuk and Stadnicka				C	Valence		3.937	O3A	1.304	1.253	
	C13	Valence	2012				C9	1.379		C10	Valence		4.339	
C8	1.404	1.505		C2	Valence	C	1.388	C4	1.408	1.487		C7	Valence	
C12	1.399	1.528	C3	1.356	1.738	O	1.378	C5	1.411	1.474	O5	1.228	1.646	
C17	1.520	1.067	C9	1.490	1.168		4.161	C9	1.405	1.500	O6	1.289	1.322	
		4.101	O1	1.347	1.074		Valence			4.461	C4	1.519	1.071	
	C6	Valence			3.981	C1	1.430		C9	Valence			4.039	
C5	1.407	1.492		C5	Valence	C	1.400	C8	1.392	1.561		C6	Valence	
C7	1.396	1.541	C4	1.458	1.284	O	1.350	C10	1.405	1.500	O3	1.242	1.565	
C11	1.522	1.061	C6	1.356	1.737		3.979	O1	1.372	0.980	O4	1.272	1.405	
		4.095	O8	1.351	1.061		Valence			4.042	C2	1.505	1.116	
	C10	Valence			4.081	C4	1.320		C2	Valence			4.086	
C5	1.411	1.474	Lee et al. 2006				C	1.400	C3	1.453	1.303		C1	Valence
C9	1.409	1.483		C9	Valence	O	1.400	O1	1.395	0.906	O5	1.245	1.548	
C14	1.517	1.077	C8	1.522	1.062		4.342	O2	1.211	1.748	O6	1.254	1.499	
		4.035	C13	1.516	1.080		Valence			3.957	C2	1.513	1.090	
	C18	Valence	C14	1.521	1.065	C6	1.400		C10	Valence			4.137	
C17	1.421	1.431	O2	1.457	0.725	C	1.380	C4	1.438	1.361		C18	Valence	
C19	1.392	1.560			3.933	O	1.340	C5	1.408	1.488	O3	1.290	1.318	
C23	1.507	1.109	Leon et al. 2002					4.241	C9	1.409	1.483	O4	1.232	1.622
		4.101		C	Valence		Valence			4.332	C16	1.496	1.146	
	C22	Valence	C1	1.386	1.588	C9	1.420		C9	Valence			4.086	
C17	1.416	1.453	C	1.387	1.583	C	1.380	C8	1.370	1.665	Lu and Peters 2006			
C21	1.380	1.616	O	1.378	0.961	O	1.360	C10	1.409	1.483		C29	Valence	
C26	1.530	1.036			4.132		4.077	O1	1.379	0.958	C28	1.484	1.188	
		4.105		C	Valence	Liu et al. 2002					4.106	C30	1.397	1.537
	C30	Valence	C4	1.387	1.583		C3	Valence		C2	Valence	C34	1.399	1.528
C29	1.419	1.440	C	1.387	1.583	C1	1.508		C3	1.442	1.345			4.252
C31	1.393	1.555	O	1.387	0.930	C4	1.211		O1	1.382	0.947		C33	Valence
C35	1.516	1.080			4.097		3.773		O2	1.206	1.781	C28	1.431	1.390
		4.075		C	Valence		Valence			4.073		C32	1.389	1.574
	C34	Valence	C1	1.378	1.626	C3	1.211		C10	Valence	C38	1.517	1.077	
C29	1.410	1.479	C	1.390	1.569	C5	1.453		C4	1.435	1.373			4.040
C33	1.386	1.588	O	1.369	0.992		3.969		C5	1.408	1.488		C29	Valence
C38	1.526	1.049			4.188		Valence		C9	1.398	1.532	C28	1.419	1.440
		4.115		C	Valence	C2	1.339			4.393		C30	1.394	1.551
Hill et al. 2007			C4	1.369	1.670	C4	1.506		C9	Valence	C35	1.508	1.106	
	C11	Valence	C	1.390	1.569	C9	1.495		C8	1.378	1.626			4.097
C10	1.383	1.602	O	1.359	1.029		4.087		C10	1.398	1.532		C29	Valence
C12	1.226	2.551			4.268		Valence		O1	1.381	0.951	C28	1.537	1.015
		4.153		C	Valence	C4	1.453			4.109		C30	1.376	1.636
	C12	Valence	C6	1.358	1.725	C6	1.401		C2	Valence	C34	1.400	1.523	
C11	1.226	2.551	C	1.377	1.631	C10	1.399		C3	1.448	1.321			4.174
C13	1.415	1.457	O	1.391	0.917		4.348		O1	1.375	0.971		C34	Valence
		4.008			4.273		Valence		O2	1.211	1.749	C29	1.400	1.523
	C3	Valence		C	Valence	C2	1.573			4.042		C33	1.376	1.636
C4	1.190	2.839	C9	1.355	1.741	C3	1.508		C10	Valence	C35	1.537	1.015	
C31	1.420	1.436	C	1.377	1.631	C11	1.529		C4	1.430	1.394			4.174
		4.274	O	1.384	0.941	O1	1.445		C5	1.406	1.496		C36	Valence
	C4	Valence			4.312		3.813		C9	1.401	1.519	C35	1.526	1.049

Bond Length – Bond Valence Relationships for C-C and C-O Bonds

C37	1.377	1.631	C2	1.407	1.492	C7	Valence	O20	1.220	1.694	C8	1.391	1.564	
C41	1.405	1.501	C9	1.415	1.457	C6	1.497	1.143	O21	1.336	4.232			
		4.180	C10	1.473	1.227	O1	1.314	1.209			C7	Valence		
	C41	Valence			4.176	O2	1.227	1.652		C2	Valence	C2	1.398	1.532
C28	1.529	1.039		C6	Valence			4.003	C3	1.458	1.284	C6	1.382	1.607
C36	1.405	1.501	C7	1.393	1.555	Mattar et al. 2004			O1	1.389	0.923	O	1.407	0.866
C40	1.372	1.655	C7#1	1.393	1.555	C13	Valence		O11	1.214	1.733			4.005
		4.195	C6#2	1.512	1.093	C5	1.394	1.550			3.941		C2	Valence
	C29	Valence			4.203	C9	1.490	1.166		C19	Valence	C1	1.503	1.123
C28	1.390	1.569	Manbeck et al. 2012			C14	1.392	1.559	C3	1.473	1.228	C3	1.396	1.541
C30	1.428	1.402		C2	Valence			4.276	O20A	1.223	1.676	C7	1.398	1.532
C34	1.435	1.373	C1	1.504	1.119		C14	Valence	O21	1.340	1.100			4.196
		4.344	C3	1.396	1.541	C8	1.396	1.543			4.005		C10	Valence
	C34	Valence	C7	1.395	1.546	C10	1.489	1.170	Pandey et al. 2014			C9	1.365	1.690
C29	1.435	1.373			4.207	C13	1.392	1.559		C6	Valence	C11	1.415	1.457
C33	1.418	1.444		C9	Valence			4.272	C7	1.491	1.163	O1	1.382	0.947
C35	1.422	1.427	C8	1.514	1.087	Mehn et al. 2003			O3	1.339	1.105			4.094
		4.244	C10	1.389	1.574		C1	Valence	O4	1.200	1.820		C11	Valence
	C36	Valence	C14	1.393	1.555	C2	1.569	0.923			4.088	C2	1.419	1.440
C35	1.453	1.302			4.216	O1	1.278	1.376		C5	Valence	C6	1.421	1.431
C37	1.405	1.501		C1	Valence	O3	1.194	1.859	C1	1.520	1.067	C10	1.415	1.457
C41	1.408	1.488	C2	1.393	1.555			4.158	O1	1.261	1.462			4.328
		4.290	C6	1.361	1.710		C1	Valence	O2	1.231	1.628		C6	Valence
	C2	Valence	C7	1.511	1.096	C2	1.493	1.156			4.157	C5	1.411	1.474
C1	1.521	1.064			4.361	O1	1.285	1.341		C18	Valence	C7	1.423	1.423
C3	1.414	1.461		C6	Valence	O2	1.240	1.576	C19	1.489	1.170	C11	1.421	1.431
C7	1.366	1.685	C1	1.414	1.461			4.074	O9	1.341	1.097			4.329
		4.210	C5	1.391	1.564		C1	Valence	O10	1.205	1.787		C21	Valence
	C7	Valence	C7	1.497	1.143	C2	1.505	1.116			4.055	C20	1.348	1.777
C2	1.366	1.685			4.169	O1	1.263	1.452		C17	Valence	C22	1.421	1.431
C6	1.374	1.645		C3	Valence	O2	1.237	1.593	C13	1.524	1.055	O2	1.404	0.875
C8	1.539	1.009	C4	1.530	1.036			4.161	O7	1.264	1.446			4.084
		4.339	O3	1.281	1.361		C1	Valence	O8	1.237	1.593		C22	Valence
	C14	Valence	O4	1.227	1.652	C2	1.528	1.042			4.095	C13	1.426	1.410
C1	1.530	1.036			4.049	O1	1.201	1.813	Reddy et al. 2014			C17	1.411	1.474
C9	1.382	1.607		C1	Valence	O2	1.251	1.515		C1	Valence	C21	1.421	1.431
C13	1.390	1.569	C2	1.517	1.077			4.371	C2	1.487	1.177			4.316
		4.212	O1	1.261	1.462	Munshi et al. 2010			O1	1.297	1.285		C17	Valence
	C21	Valence	O2	1.237	1.593		C3	Valence	O2	1.203	1.800	C16	1.406	1.496
C16	1.317	1.948			4.132	C2	1.454	1.299			4.262	C18	1.425	1.414
C20	1.425	1.414		C8	Valence	C4	1.379	1.620	Saeed et al. 2012			C22	1.411	1.474
C22	1.552	0.971	C9	1.514	1.087	C19	1.480	1.204		C11	Valence			4.385
		4.333	O3	1.291	1.313			4.122	C1	1.455	1.293		C11	Valence
	C16	Valence	O4	1.225	1.663		C8	Valence	C15	1.355	1.742	C10	1.361	1.710
C15	1.623	0.787			4.063	C7	1.437	1.364	O12	1.362	1.017	C12	1.425	1.414
C17	1.371	1.660		C1	Valence	C9	1.505	1.116			4.052	O1	1.391	0.917
C21	1.317	1.948	C2	1.504	1.119	C18	1.423	1.423		C31	Valence			4.042
		4.395	O1	1.278	1.376			3.903	C2	1.459	1.280		C12	Valence
	C23	Valence	O2	1.242	1.565		C3	Valence	C35	1.357	1.730	C3	1.420	1.436
C22	1.543	0.997			4.060	C2	1.458	1.284	O32	1.371	0.985	C7	1.417	1.448
C24	1.346	1.788		C1	Valence	C4	1.382	1.607			3.994	C11	1.425	1.414
C28	1.401	1.519	C2	1.501	1.129	C19	1.473	1.228	Schumann et al. 2003					4.299
		4.304	O1	1.291	1.313			4.119		C14	Valence		C7	Valence
	C28	Valence	O2	1.228	1.646		C8	Valence	C9	1.376	1.636	C6	1.406	1.496
C15	1.524	1.055			4.088	C7	1.438	1.361	C13	1.381	1.612	C8	1.418	1.444
C23	1.401	1.519		C3	Valence	C9	1.506	1.112	O	1.435	0.783	C12	1.417	1.448
C27	1.367	1.680	C4	1.506	1.113	C18	1.419	1.438			4.030			4.389
		4.253	O1	1.336	1.117			3.911		C8	Valence		C22	Valence
Maher et al. 2010			O2	1.203	1.800		C2	Valence	C3	1.391	1.564	C21	1.361	1.710
	C10	Valence			4.030	C3	1.454	1.299	C7	1.378	1.626	C23	1.420	1.436
C1	1.473	1.227		C7	Valence	O1	1.392	0.915	O	1.404	0.875	O2	1.390	0.920
O1	1.348	1.070	C1	1.511	1.096			4.066			4.066			4.066
O2	1.211	1.749	O1	1.280	1.366	O11	1.211	1.747		C3	Valence		C23	Valence
		4.046	O2	1.221	1.688			3.960	C2	1.502	1.126	C14	1.429	1.398
	C1	Valence			4.149		C19	Valence	C4	1.396	1.541	C18	1.412	1.470
						C3	1.480	1.204						

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C22	1.420	1.436			3.921	O2	Valence		O11	Valence	C2	1.151	2.169		
		4.304		C25	Valence	C2	1.145	2.216	C2	1.211	1.747		2.169		
	C18	Valence		C26	1.522			2.216			1.747	O1	Valence		
C17	1.438	1.361		C27	1.523		O3	Valence		O20	Valence	C1	1.115	2.468	
C19	1.424	1.419		C28	1.515		C3	1.170	2.026	C19	1.220	1.694	2.468		
C23	1.412	1.470		O3	1.444			2.026			1.694	O2	Valence		
		4.250			3.961		Krawczuk and Stadnicka 2012			O11	Valence	C2	1.145	2.216	
	C9	Valence		C21	Valence				C2	1.214	1.733		2.216		
C8	1.327	1.891		C22	1.531		O1	Valence			1.733	O1	Valence		
C10	1.435	1.373		C23	1.526		C2	1.347	1.074		O20A	Valence	C1	1.146	2.208
O	1.389	0.924		C24	1.527		C6	1.360	1.026	C19	1.223	1.676	2.208		
		4.188		O1	1.414			2.100			1.676	O2	Valence		
	C10	Valence			3.972					O1	Valence	C2	1.144	2.224	
C1	1.435	1.373		Oxygen Environments			C4	1.248	1.532	C2	1.392	0.915	2.224		
C5	1.413	1.466		Bleeke et al. 2010				1.532		C9	1.369	0.992	O1	Valence	
C9	1.435	1.373					Liu et al. 2013				1.907	C1	1.140	2.256	
		4.212		O1	Valence		O1	Valence		O21	Valence		2.256		
	C5	Valence		C4	1.385		C2	1.373	0.978	C19	1.336	1.117	O2	Valence	
C4	1.416	1.453		C5	1.428		C9	1.386	0.934	C22	1.445	0.757	C2	1.160	2.100
C6	1.406	1.496			1.740			1.912			1.874		2.100		
C10	1.413	1.466		O1	Valence		O1	Valence		O1	Valence	Reddy et al. 2014			
		4.415		C1	1.148		C2	1.395	0.906	C2	1.389	0.923	O2	Valence	
Song et al. 2007					2.197		C9	1.372	0.980	C9	1.372	0.982	C1	1.203	1.800
	C5	Valence			2.197			1.886			1.905		1.800		
C4	1.340	1.820		O2	Valence		O1	Valence		O21	Valence	O3	Valence		
C6	1.406	1.496		C2	1.149		C2	1.382	0.946	C19	1.340	1.100	C3	1.229	1.640
C13	1.479	1.205			2.189		C9	1.379	0.956	C22	1.443	0.762		1.640	
		4.522		Bleeke and Anutrasakda 2012				1.902		Pandian et al. 2010		Saeed et al. 2012			
	C1	Valence		O1	Valence		O1	Valence		O1	Valence	O12	Valence		
O1	1.172	2.012		C9	1.156		C2	1.375	0.971			C11	1.362	1.017	
O2	1.362	1.018			2.133		C9	1.377	0.964	C9	1.361	1.021	C13	1.359	1.030
O3	1.303	1.258			2.133			1.936		C25	1.436	0.780		2.047	
		4.287		O5	Valence		O1	Valence			1.802	O11	Valence		
	C2	Valence		C59	1.156		C2	1.380	0.954		1.802	C1	1.210	1.754	
		4.147			2.131		C9	1.377	0.964	C16	1.381	0.951		1.754	
O2	1.341	1.097		O1	Valence			1.919		C29	1.455	0.729	O21	Valence	
O4	1.337	1.113		C9	1.116		Lu and Peters 2006				1.680	C2	1.218	1.708	
C6	1.319	1.937			2.459		O1	Valence		O3	Valence		1.708		
		4.147		Draskovic et al 2010			C28	1.073	2.869	C17	1.331	1.137	Schumann et al. 2003		
Varga et al. 2009				O1	Valence			2.869		C33	1.444	0.758	O	Valence	
	C29	Valence		C1	1.427			2.869			1.896	C14	1.435	0.783	
C30	1.515	1.083		C2	1.364		O2	Valence			1.896	C15	1.446	0.753	
C31	1.521	1.064			1.817		C29	1.146	2.208		0.992		1.536		
C32	1.521	1.064		Esteruelas et al. 2000				2.208		C24	1.369	0.698	Song et al. 2007		
O4	1.440	0.769		O3	Valence		O3	Valence		C37	1.467	1.691	O1	Valence	
		3.981		C9	1.156		C30	1.147	2.200		1.691		C1	1.325	1.162
	C21	Valence			2.131			2.200		Pierce et al. 2009		C5	1.414	0.845	
C22	1.526	1.049		Huynh et al. 2005			O4	Valence		O1	Valence		2.007		
C23	1.524	1.055		O1	Valence		C31	1.139	2.265	C1	1.151	2.169			
C24	1.525	1.052		C1	1.162			2.265			2.169				
O2	1.441	0.766			2.085		Munshi et al. 2010			O2	Valence				
					2.085										

Bond Length – Bond Valence Relationships for C-C and C-O Bonds

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Wave Profile for Anti-force Waves with Maximum Possible Currents

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Running Title: Wave Profile for Anti-force Waves with Maximum Possible Currents

Abstract

In the theoretical investigation of the electrical breakdown of a gas, we apply a one-dimensional, steady state, constant velocity, three component fluid model and consider the electrons to be the main element in propagation of the wave. The electron gas temperature, and therefore the electron gas partial pressure, is considered to be large enough to provide the driving force. The wave is considered to have a shock front, followed by a thin dynamical transition region. Our set of electron fluid-dynamical equations consists of the equations of conservation of mass, momentum, and energy, plus the Poisson's equation. The set of equations is referred to as the electron fluid dynamical equations; and a successful solution thereof must meet a set of acceptable physical conditions at the trailing edge of the wave.

For breakdown waves with a significant current behind the shock front, modifications must be made to the set of electron fluid dynamical equations, as well as the shock condition on electron temperature. Considering existence of current behind the shock front, we have derived the shock condition on electron temperature, and for a set of experimentally measured wave speeds, we have been able to find maximum current values for which solutions to our set of electron fluid dynamical equations become possible. We will present the wave profile for electric field, electron velocity, electron temperature, and electron number density within the dynamical transition region of the wave.

Introduction and Model

The electrical breakdown of a gas in the presence of a high electric field occurs by a wave propagating with speeds approaching the speed of light and a discontinuity at the wave front. The most common form of breakdown waves in nature is lightning strokes of varying classifications. The basic model consists of

a volume of excess charge (electron gas) advancing into a neutral gas, partially ionizing the neutral gas. In anti-force waves (Lightning return strokes), the direction of the electric field force on the electrons causes an electron mobility motion in the opposite direction of the wave propagation. However, the electron gas temperature, and therefore the electron gas partial pressure, is large enough to provide the net force for propagation of the wave. Return strokes in lightning can consist of a wave for which a large current exists behind the shock front.

The basic set of equations which apply to electron gas alone, consists of the equations of conservation of mass, momentum and energy; and the Maxwell's equations reduces to the Poisson's equation alone. The wave front is considered to be a shock front, followed by a thin dynamical transition region, referred to as the sheath region of the wave. In the sheath region, the electric field and electron velocity are changing rapidly; however, the changes in the electron number density and temperature are not so rapid by comparison. The sheath region is followed by a relatively thicker region, referred to as the quasi-neutral region of the wave; where in this region the electric field is zero, and because of further ionization of heavy particles, the electron gas cools down to room temperature and the electrons slow down to speeds comparable to those of heavy particles.

For theoretical investigation of breakdown waves, we employ the set of equations developed by Fowler et al. (1984). Their set of equations which describe pro-force waves consists of the equations of conservation of mass, momentum, and energy coupled with the Poisson's equation. The set of equations respectively are

$$\frac{d(nv)}{dx} = n\beta, \quad (1)$$

$$\frac{d}{dx}[mnv(v-V) + nkT_e] = -enE - Kmn(v-V), \quad (2)$$

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$$\frac{d}{dx} [mnv(v-V)^2 + nkT_e(5v-2V) + 2env\Phi - \frac{5nkT_e}{mK} \frac{dT_e}{dx}] = -3\left(\frac{m}{M}\right)nkKT_e - \left(\frac{m}{M}\right)Kmn(v-V)^2, \quad (3)$$

$$\frac{dE}{dx} = \frac{e}{\varepsilon_0} n \left(\frac{v}{V} - 1 \right). \quad (4)$$

Where v, n, T_e, m and e represent electron velocity, number density, temperature, mass and charge respectively. $E_0, E, V, M, K, k, \beta, \phi, x$, represent electric field at the shock front, electric field within the sheath region of the wave, wave velocity, neutral particle mass, elastic collision frequency, Boltzmann's constant, ionization frequency, ionization potential of the gas and position within the sheath region of the wave respectively.

To be able to integrate the set of equations (1-4) through the sheath region of the wave, Fowler et al. (1984) reduced the set of equations to non-dimensional form. Fowler et al's (1984) set of non-dimensional variables are

$$\eta = \frac{E}{E_0}, \nu = \left(\frac{2e\phi}{\varepsilon_0 E_0^2} \right) n, \psi = \frac{v}{V}, \theta = \frac{T_e k}{2e\phi}, \xi = \frac{eE_0 x}{mV^2},$$

$$\alpha = \frac{2e\phi}{mV^2}, \kappa = -\frac{mV}{eE_0} K, \mu = \frac{\beta}{K}, \omega = \frac{2m}{M},$$

where $\eta, \nu, \psi, \theta, \mu$ and ξ represent the non-dimensional electric field, electron number density, electron velocity, electron gas temperature, ionization rate, and position within the sheath region of the wave, respectively; while α and κ represent wave parameters. Substituting the non-dimensional variables in equations (1-4), the set of equations become

$$\frac{d(\nu\psi)}{d\xi} = \kappa\mu\nu, \quad (5)$$

$$\frac{d}{d\xi} [\nu\psi(\psi-1) + \alpha\nu\theta] = -\nu\eta - \kappa\nu(\psi-1), \quad (6)$$

$$\frac{d}{d\xi} [\nu\psi(\psi-1)^2 + \alpha\nu\theta(5\psi-2) + \alpha\nu\psi + \alpha\eta^2 - \frac{5\alpha^2\nu\theta}{\kappa} \frac{d\theta}{d\xi}] = -\omega\kappa\nu[3\alpha\theta + (\psi-1)^2], \quad (7)$$

$$\frac{d\eta}{d\xi} = \frac{\nu}{\alpha} (\psi-1). \quad (8)$$

In the case of theoretical investigation of anti-force waves, we employ the set of dimensionless variables developed by Hemmati (1999); in which all quantities including κ are positive, and the position within the sheath, ξ , is positive backward. Hemmati's (1999) set of dimensionless variables for anti-force waves, which have proven to be successful are

$$\eta = \frac{E}{E_0}, \nu = \left(\frac{2e\phi}{\varepsilon_0 E_0^2} \right) n, \psi = \frac{v}{V}, \theta = \frac{T_e k}{2e\phi}, \xi = -\frac{eE_0 x}{mV^2},$$

$$\alpha = \frac{2e\phi}{mV^2}, \kappa = -\frac{mV}{eE_0} K, \mu = \frac{\beta}{K}, \omega = \frac{2m}{M}.$$

For anti-force waves (lightning return strokes) with a large current behind the wave front, in addition to the equation of conservation of energy and the Poisson's equation, the boundary condition on electron temperature at the shock front must be modified as well. For anti-force waves with a large current behind the wave front, we will use Hemmati et. al's (2011) modified set of non-dimensional equations.

$$\frac{d}{d\xi} [\nu\psi] = \kappa\mu\nu, \quad (9)$$

$$\frac{d}{d\xi} [\nu\psi(\psi-1) + \alpha\nu\theta] = \nu\eta - \kappa\nu(\psi-1), \quad (10)$$

$$\frac{d}{d\xi} [\nu\psi(\psi-1)^2 + \alpha\nu\theta(5\psi-2) + \alpha\nu\psi - \frac{5\alpha^2\nu\theta}{\kappa} \frac{d\theta}{d\xi} + \alpha\eta^2] = 2\eta\kappa\alpha - \omega\kappa\nu[3\alpha\theta + (\psi-1)^2], \quad (11)$$

$$\frac{d\eta}{d\xi} = \kappa\mu - \frac{\nu}{\alpha} (\psi-1). \quad (12)$$

Where, with I_1 representing the current behind the wave front,

$$\iota = \frac{I_1}{\varepsilon_0 K E_0}, \quad (13)$$

is the dimensionless current behind the wave front. We will use Hemmati et al's (2011) modified boundary condition on electron temperature at the shock front as well

$$\theta_1 = \frac{\psi_1(1-\psi_1)}{\alpha} - \frac{\kappa\iota}{\nu_1}. \quad (14)$$

Where, θ_1, ν_1 and ψ_1 , represent the electron temperature, number density and velocity values at the shock front.

Results and Discussion

Examining characteristics of the initial stage in lightning initiated from tall objects and in rocket-triggered lightning, Miki et al. (2005) report mean peak current of 8.8 kA for return stroke current pulses; however, they also reported lightning currents as high as 150 kA measured at the Fukui thermal plant station in Japan. In a study of propagation characteristics of lightning leaders and return strokes, Olsen et al. (2006) report rocket-triggered lightning currents typically in the range of 1 kA; however, triggered or natural lightning subsequent stroke currents are in the range of 10-15 kA. In a review of characteristics of lightning discharges that transport either positive charge or both negative and positive charges to ground, Rakov (2000) reported positive return stroke (positive flashes) currents in excess of 10 kA, an order of magnitude larger than for negative flashes. Rakov (2000) also reported direct current measurements of three positive lightning discharges in Japan with very large peaks of 340, 320, and 280 kA of initial pulses. In his review article, Rakov (2000) reported return stroke speeds in the range of $0.3 \times 10^8 \text{ m/s}$ to $1.7 \times 10^8 \text{ m/s}$. However, imaging of upward positive leaders in two artificially-initiated lightning flashes, Yoshida et al. (2010) report average steady current of 2 kA, and a peak current value of 18 kA; where their reported speed was on the order of 10^6 m/s .

Determining the ratio of the elastic collision frequency, K (McDaniel 1964), to the electron gas pressure, P , gives $K/P = 3 \times 10^8$ for helium and $K/P = 4.8 \times 10^7$ for nitrogen at 273 K. At a temperature of 10^5 K , this will be 2.4×10^9 for helium and 9×10^9 for nitrogen and applied fields are usually of the order 10^5 V/m . Considering that E_0, K, β in our formulas are scaled with P (the electron gas pressure) and using the values of I_1, ϵ_0, E_0, K one can estimate the value of ι , which is of order one.

We use a numerical trial-and-error method to integrate equations 9 - 12 through the sheath region of the wave. For a given wave speed, α , a set of values for wave constant, κ , electron velocity ψ_1 and electron density, v_1 at the wave front are chosen. The values of κ , ψ_1 and v_1 are repeatedly changed in integrating equations 9 -12 through the sheath region of the wave, until the process leads to a satisfactory conclusion meeting the expected physical conditions at the end of the sheath region of the wave.

$$\left[\eta_2 \rightarrow 0, \psi_2 \rightarrow 1, \left(\frac{d\eta}{d\xi} \right)_2 \rightarrow 0, \left(\frac{d\psi}{d\xi} \right)_2 \rightarrow 0 \right]. \quad [15]$$

For $\alpha = 0.001, 0.01, 0.1$ and 1 , we have been able to integrate the set of electron fluid dynamical equations (equations 9-12) for maximum non-dimensional current, ι , values of $7, 5, 1$ and 0.5 respectively, for which solutions to the set of equations became possible and met the expected physical conditions at the trailing edge of the wave. $\alpha = 0.001$ represents a fast wave speed of $0.937 \times 10^8 \text{ m/s}$ and $\alpha = 1$ represents a very low wave speed of $0.3 \times 10^7 \text{ m/s}$ for lightning return strokes. In a review of available experimental data on return-stroke speed for both negative and positive lightning, minimum return-stroke speed reported by Rakov (2007) was $2 \times 10^7 \text{ m/s}$.

The successful solutions for the indicated speeds, α , and dimensionless currents, ι , required the following boundary values

$$\alpha = 0.001, \iota = 7, \kappa = 0.144, \psi_1 = 0.472, v_1 = 0.2161$$

$$\alpha = 0.01, \iota = 5, \kappa = 1.3, \psi_1 = 0.7, v_1 = 0.7696$$

$$\alpha = 0.1, \iota = 1, \kappa = 0.44, \psi_1 = 0.832, v_1 = 0.71$$

$$\alpha = 1, \iota = 0.5, \kappa = 0.1338, \psi_1 = 0.5502, v_1 = 0.4882$$

Figure 1 represents dimensionless electric field, η , as a function of dimensionless electron velocity, ψ , within the sheath region of the wave. For all dimensionless wave speed and current values, our solutions meet the expected conditions at the end of the sheath region of the wave. Upon close inspection of the curve with $\alpha = 0.001$ and $\iota = 7$, it seems that the electric field value at the shock front starts at 3; although that is not the case. During integration of the set of electron fluid dynamical equations through the sheath region, particularly for slower wave speeds and larger current values, the sheath thickness become relatively large, making the number of data points also very large. To keep it uniform for all wave speeds and current values, only one out of every ten data points is print. Therefore, the first point indicated in the curve for $\alpha = 0.001$ and $\iota = 7$, is actually the tenth data point. However, as was indicated, we have been able to solve the set of equations for lower speeds than those observed experimentally. Also, for large current values and slow wave speeds, integration of the set of electron fluid dynamical equations through the sheath region of the wave becomes very time consuming and difficult.

Wave Profile for Anti-force Waves with Maximum Possible Currents

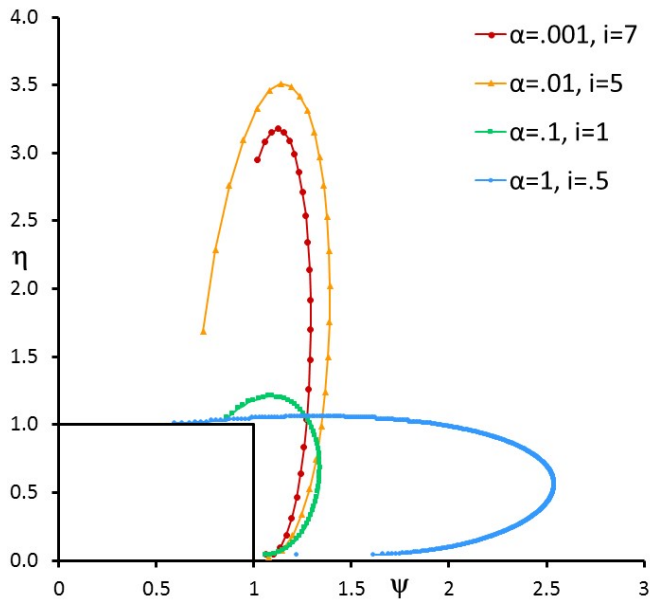


Figure 1: Electric field, η , as a function of electron velocity, ψ , within the sheath region of anti-force current bearing waves for wave speed values of $\alpha = 0.001, 0.01, 0.1, 1$, and maximum dimensionless current values of 7, 5, 1, and 0.5, for which solutions to the set of electron fluid dynamical equations became possible.

Figure 2 represents dimensionless electric field, η , as a function of dimensionless position, ξ , within the sheath region of the wave for anti-force current bearing waves. The curve for $\alpha = 1$ and $i = 5$, shows that the sheath thickness is much larger than that for $\alpha = 0.001$ and $i = 7$.

Figure 3 represents the dimensionless electron velocity, ψ , as a function of dimensionless position, ξ , within the sheath region, for anti-force current bearing waves. For all α values, solutions of the set of electron fluid dynamical equations met the expected boundary conditions at the trailing edge of the wave; nevertheless, for $\alpha = 1$ and $i = 0.5$, in integration of the set of equations through the sheath region of the wave, the best value we could find ψ_2 was 1.22.

Figure 4 represents electron gas temperature, θ , as a function of position, ξ , within the sheath region of anti-force current bearing waves. As α decreases and wave speed increases, the electron gas temperature becomes very large. For $\alpha = 0.1$, meaning wave speed of 9.37×10^6 m/s, our dimensionless electron temperature of approximately 1, corresponds to a temperature of 5.8×10^5 K. For the wave speed of 9.37×10^6 m/s, our electron temperature value within the sheath region of the wave, agrees well with the electron temperature reported by Sanmann (1975) for an anti-force wave with a similar wave speed.

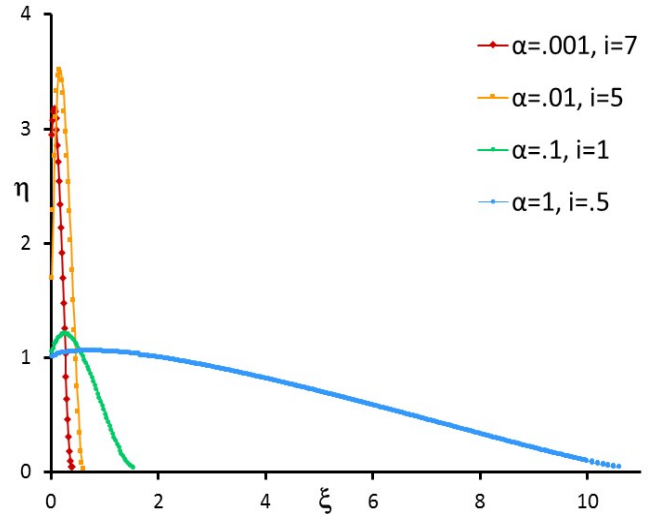


Figure 2: Electric field, η , as a function of position, ξ , within the sheath region of anti-force current bearing waves for wave speed values of $\alpha = 0.001, 0.01, 0.1, 1$, and maximum dimensionless current values of 7, 5, 1, and 0.5, for which solutions to the set of electron fluid dynamical equations became possible.

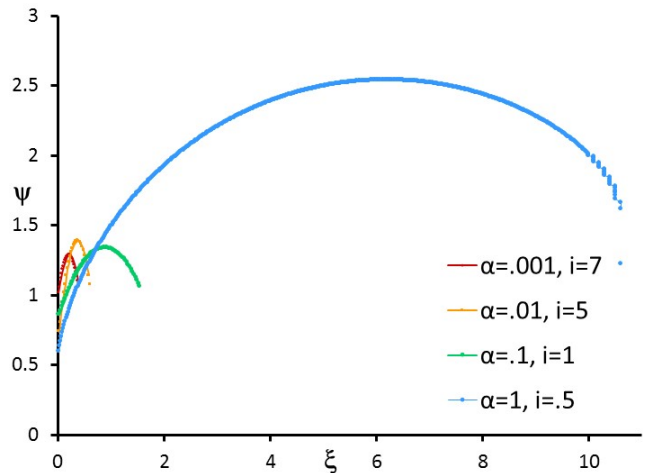


Figure 3: Electron velocity, ψ , as a function of position, ξ , within the sheath region of anti-force current bearing waves for wave speed values of $\alpha = 0.001, 0.01, 0.1, 1$, and maximum dimensionless current values of 7, 5, 1, and 0.5, for which solutions to the set of electron fluid dynamical equations became possible.

Figure 5 represents dimensionless electron number density, v , as a function of position within the sheath region of anti-force current bearing waves. For $\alpha = 0.1$ and wave speed of 9.37×10^6 m/s, our average dimensionless electron number density of 0.6, corresponds with electron number of 7.7×10^{15} electrons/m³. Again, for a wave speed of 9.37×10^6 m/s, our electron number density value compares well with the electron number density for an anti-force wave of

similar wave speed reported by Sanmann (1975).

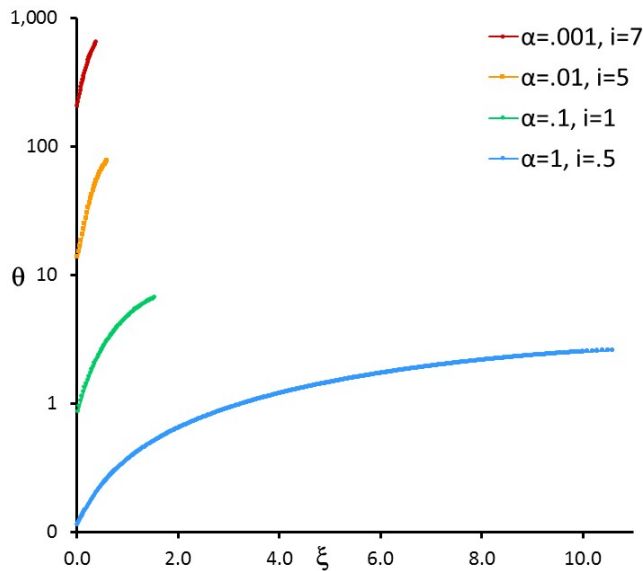


Figure 4: Electron temperature, θ , as a function of position, ξ , within the sheath region of anti-force current bearing waves for wave speed values of $\alpha = 0.001, 0.01, 0.1, 1$, and maximum dimensionless current values of 7, 5, 1, and 0.5, for which solutions to the set of electron fluid dynamical equations became possible.

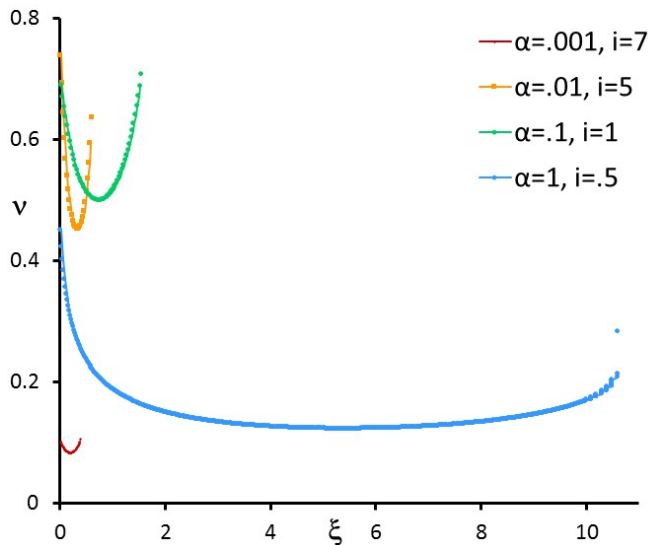


Figure 5: Electron number density, v , as a function of position, ξ , within the sheath region of anti-force current bearing waves for wave speed values of $\alpha = 0.001, 0.01, 0.1, 1$, and maximum dimensionless current values of 7, 5, 1, and 0.5, for which solutions to the set of electron fluid dynamical equations became possible.

Conclusions

For current bearing anti-force waves, we have been able to solve our set of electron fluid dynamical equations for the range of current values reported by experimentalists. In fact, we have been able to integrate the set of equations for larger current values, which have been reported by a few experimentalists as well; indicating that in lightning return strokes, such large currents may exist. Also, we have been able to integrate our set of electron fluid dynamical equations for much lower speeds than those reported experimentally, implying that return strokes with lower wave speeds should be detected as well. For anti-force current bearing waves, as wave speed increases, the current values for which solutions for the set of electron fluid dynamical equations become possible increases as well. As the wave speed decreases and the current value increases, integration of the set of electron fluid dynamical equations through the sheath region of the wave becomes time consuming and difficult.

Acknowledgement

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Aquatic Effects of a Localized Oil Spill on Lake Conway, AR and Its Tributaries

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Running title: Localized Oil Spill on Lake Conway, AR and Tributaries

Abstract

Oil spills, no matter where they occur, elicit environmental concern and avoiding these disasters should be a priority. Old pipelines that are not regularly maintained and carry large amounts of crude oil long distances are of particular concern. One such pipeline is the 65 year-old Pegasus pipeline owned by ExxonMobil. On March 29, 2013, 795,000 L of Wabasca Heavy Canadian crude oil spilled into a neighborhood of Mayflower, Arkansas, when the Pegasus pipeline ruptured. This spill led to the evacuation of many homes in the surrounding neighborhood. Drainage ditches in the affected neighborhood drained oil into a nearby cove of Lake Conway. This lake is popular for recreational fishing, thus concerns were raised not only about the potential effects of the oil spill on area residents, but also the lake and its biological communities. Ultimately, this project assessed the effect of the oil spill in water and sediment samples on freshwater test organisms. Samples were collected at 6 sites in the affected neighborhood and in Lake Conway. Chronic Whole Effluent Toxicity (WET) tests were performed on water samples using *Pimephales promelas* and *Ceriodaphnia dubia*. Acute sediment toxicity tests were performed using *Chironomus dilutus*. These tests measured sub-lethal toxicity in at least one of the sampled sites, indicating that further investigation of environmental after-effects is warranted.

Introduction

On March 29, 2013, a 6.71 meter rupture occurred in the 65 year-old Pegasus pipeline running through Mayflower, Arkansas, spilling 795,000 L of Wabasca Heavy Canadian Crude oil into a nearby neighborhood (Gallucci 2013b). This led to the evacuation of many homes and many complaints of sickness ranging from nausea to bronchitis. Spilled oil also reached a cove of nearby Lake Conway, a 2,700 hectare recreational

fishing lake (Gallucci 2013a). Remediation began immediately after the spill and concluded with the affected cove being completely dredged. This action included removal by vacuuming the oil and contaminated water, excavation of contaminated vegetation and soil, and blocking the flow of water from the cove to the main body of the lake (Hardy 2013).

The extension of hook cracks was responsible for the rupture in the Pegasus pipeline (Douglas 2013). These cracks are common in old pipelines, however, the cracks in the Pegasus pipeline probably grew because of high pressure swings due to the type of oil the pipeline was carrying. At the time of the rupture, the Pegasus pipeline was carrying Wabasca Heavy Canadian crude oil, a form of diluted bitumen or dilbit, which is heavy and possibly made the pressure swings harder to push through the pipeline (Douglas 2013). Dilbit also could have contributed to the increase of hydrogen atoms moving to the fragile hook cracks of the pipeline. This type of crude oil contains the second-highest sulfur content of 29 types of Canadian crude oil (Douglas 2013). When hydrogen sulfide decomposes, it releases hydrogen atoms which move to fragile seams in pipelines and increases stress.

Dilbit not only causes harm inside pipelines, but also poses a great risk to humans and the environment due mainly to its harmful chemical makeup. The United States Environmental Protection Agency (USEPA) and United States Coast Guard (USCG) rank petroleum-based oil on a scale from 1-5. Group 1 includes gasoline or kerosene, having a density of less than 0.8, while group 5 includes crudes having a density greater than 1 (POLARIS 2013). Dilbit can be found in group 2, having a density of 0.85-0.95, higher than gas oil and light crudes (POLARIS 2013). The greater the density of the oil, the more likely it is to sink into the water column or sediment, increasing the chance of harm done to surrounding organisms.

Total petroleum hydrocarbons (TPH) are a mixture of several hundred chemicals that are found in crude oil (ATSDR 1999). Instead of focusing on each

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individual chemical, TPHs compile all of these chemicals, including hexane, toluene, xylenes, and naphthalene (ATSDR 1999). TPH exposure could cause nervous system issues such as headaches and dizziness (ATSDR 1999). In aquatic environments, TPH can sink to the bottom or float and may remain in soil for long periods of time (ATSDR 1999).

Dilbit is composed of benzene, polycyclic aromatic hydrocarbons (PAHs), and several heavy metals such as vanadium and arsenic (Swift et al. 2011). PAHs are cause for concern due to their environmental persistence and recalcitrant nature in water (USEPA 2008). In humans, acute exposure to benzene and PAHs have been shown to cause respiratory, gastrointestinal, and neurological problems, while long term exposure has been known to cause cancer (Swift et al. 2011). Heavy metals, such as vanadium and arsenic, are not biodegradable, accumulate in the environment, and are hazardous to humans and wildlife (Swift et al. 2011). Based on these possible effects, a dilbit spill should not be taken lightly, which is why action occurred immediately to remediate the effects of the spill.

ExxonMobil and the Arkansas Department of Environmental Quality (ADEQ) collected daily water and air samples in the days following the spill (ADEQ 2013). Sediment samples were collected at a later date, allowing time for any remaining chemicals to settle. Samples were analyzed extensively for the presence of a variety of chemicals commonly associated with oil spills as mentioned previously, including arsenic (As), chromium (Cr), lead (Pb), vanadium (Vd), and PAHs including benzo(a)anthracene, benzo(a)pyrene, and pyrene. However, no whole effluent toxicity (WET) or sediment toxicity tests were performed to determine the potential threat to resident organisms (ADEQ 2013). Therefore, the research in this study by ASU Ecotoxicology Research Facility (ERF) included WET and sediment toxicity testing to determine if there was any measured toxicity that could possibly be linked to the spill.

Aquatic organisms used in this study include *Ceriodaphnia dubia*, *Pimephales promelas*, and *Chironomus dilutus*, exposed to water and sediment respectively. All of these organisms are regularly used in toxicity testing for many reasons. They are easily cultured in the laboratory (ASU ERF), sensitive to many different pollutants, and are generally available throughout the year (USEPA 2002). The fact that these organisms are susceptible to a variety of pollutants makes them very suitable for use in toxicity testing.

All of these chemicals are to some extent toxic to aquatic organisms. Benzo(a)anthracene is the most toxic of the three PAHs with a lethal concentration at 50 percent (LC₅₀) of 10 µg/L when exposed to *Daphnia pulex* (a standard aquatic test organism) for four days (USEPA, 2014). Pyrene, is the next toxic of the three PAHs with an LC₅₀ of 135.8 µg/L and an effective concentration at 50 percent (EC₅₀) for growth of 72.7 µg/L when exposed to *Daphnia magna* (USEPA 2014). While still toxic, benzo(a)pyrene has the least toxicity of the three PAHs with a LC₅₀ of 250 µg/L when exposed to *D. pulex*.

While PAHs are more toxic overall to aquatic test organisms compared to the other chemicals, the metals that were analyzed in this study are also harmful to aquatic organisms at high concentrations. Toxic effects of metals vary between species such as *D. magna* and *Hyallela azteca* (aquatic sediment organism). The range of toxicity of the metals when *D. magna* were exposed to them for a 48-h acute test are as follows (greatest to lowest toxicity): Cr (22 µg/L), Vd (1550 µg/L), As (3800 µg/L) and Pb (4400 µg/L). The ranges of toxicity for the metals when they are exposed to *H. azteca* for a 7-d acute test are somewhat different: Pb (20 µg/L), As (426 µg/L), Vd (1251 µg/L) and Cr (>3150 µg/L) (USEPA 2014). Due to the potential toxicity of these chemicals, extensive remediation should take place after spills of this nature occur.

The purpose of this project was to perform bioassays on *Pimephales promelas*, *Ceriodaphnia dubia*, and *Chironomus dilutus* to determine if there was any measurable toxicity in the areas closest to location of the spill. Bioassays were performed using water and sediment samples from six sites in and around Lake Conway. Aquatic and sediment toxicity testing utilizes surrogate organisms with known toxic endpoints to assess the impact of the oil spill on the surrounding environment. This process will determine if the remediation protocols enacted were appropriate and/or sufficient to minimize environmental impacts.

If toxic chemicals are measured in water or sediment then aquatic organisms are also predicted to have greater mortality, slower growth, and decreased reproductive output. Therefore, the hypothesis of this project is that the areas proximal to the location of the oil spill will have greater measured toxicity than areas farther away. The results of the toxicity tests were compared to the results of the chemical analyses performed by ADEQ and ExxonMobil at sites in close geographical proximity to those sampled for toxicity testing.

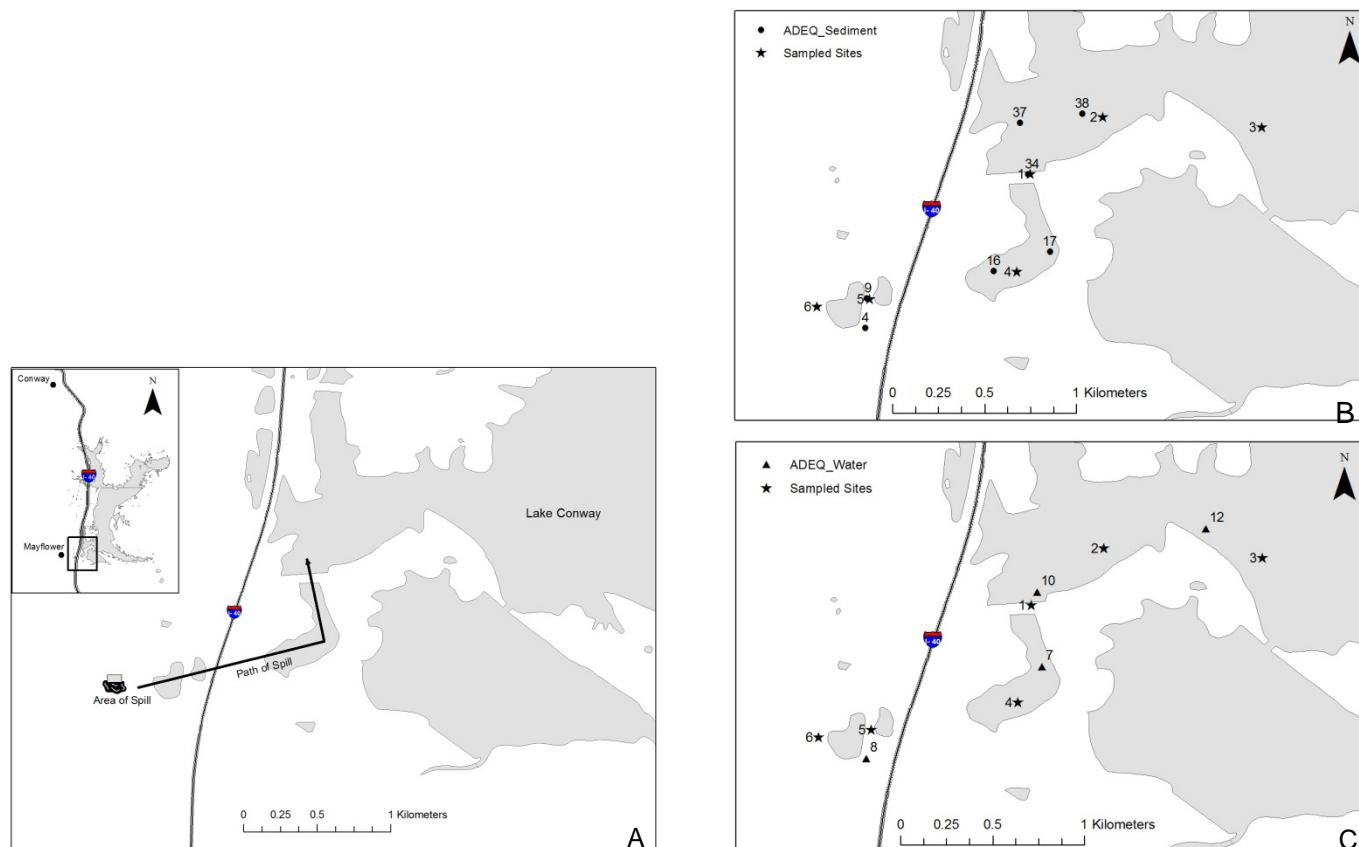


Figure 1. (A) Depicts area of spill in Mayflower, AR including location and pathway of spill from housing division through cove of Lake Conway. (B) Map depicting sites sampled by ASU with corresponding ADEC sediment sampling sites. (C) Map depicting sites sampled by ASU with corresponding ADEC water sampling sites.

Methods and Materials

Water and sediment samples were collected from 6 sites near the affected area, as well as in Lake Conway corresponding to sites sampled by ExxonMobil and Arcadis. ASU sampled for water and sediment on June 7, 2013 and again on September 11, 2013. Sites 1, 2, and 3 were located in Lake Conway and were accessed by boat: Site 1 was inside one of the barrier booms (also the location of water entry from the cove), Site 2 was in the main channel, and Site 3 was outside of the main channel of the lake and served as the lake control site. The other sites were located out of the main body of the lake: Site 4 was located in the cove of Lake Conway (the area where water, sediment and vegetation were removed for remediation), Site 5 was a ditch collecting water from the neighborhood of the oil spill and lastly, Site 6 was located in a drainage ditch immediately upstream from the affected cove (Figure 1).

The results obtained from the WET and sediment toxicity testing were compared to that of analytical testing done on water and sediment samples by

ExxonMobil and Arcadis (ADEC 2013). Water samples were collected approximately every day for an extended period of time following the oil spill by ExxonMobil and Arcadis. Therefore, the first sampling date for this study (6/7/2013) can accurately be compared to that of the work done by these organizations. However, there were no samples collected by the agencies on the second sampling date so the closest date was used for comparison. The sediment sampling done by ExxonMobil and Arcadis was not performed until July and August following the spill, serving as the only data to compare with the sediment bioassay results in this study. The sites that were sampled in this study were correlated as closely as possible to the sites sampled by ExxonMobil and Arcadis in order to best compare the data measured for each study. The data provided by ExxonMobil and Arcadis was compared to the data measured in this study by relating the amount of certain chemicals in the water and sediment to that of toxicity measured in the aquatic organisms.

Several chemicals were tested by the agencies;

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however, those that do not occur naturally in water and sediment, such as the amount of TPHs and PAHs were specifically chosen to correlate the two research studies. The chemicals that were chosen to compare to the data measured in this study are listed in Tables 2 and 3.

Water samples for our study were collected from the water column and stored in 10-L containers (USEPA 2002). The sediment samples were taken from the top 2-3 centimeters at each site and were stored in plastic Ziploc bags (USEPA 2000). The samples were put on ice and taken back to the ERF at ASU for WET and sediment toxicity testing.

Chronic (7-day) WET testing followed the United States Environmental Protection Agency (USEPA 2002) protocol. Synthetic, moderately-hard water prepared in the ERF (according to EPA standards) was used as the control for each test. WET tests were conducted with *P. promelas* (measuring survival and growth) and *C. dubia* (survival and reproduction). Following the USEPA protocol, 5 replications of 8 fish (40 fish per beaker) and 10 replications of 1 *C. dubia* were used for each WET test (USEPA 2002). Acute sediment toxicity testing was conducted using *C. dilutes* (survival and growth), also following the USEPA method (USEPA 2000). Acute testing consists of using 6 replicates of 10 chironomids, following USEPA protocol (2000). Black River sediment was used as the control, as it has been determined to be suitable for use in reference sediment toxicity testing for the Arkansas Delta ecoregion (Moore et al. 1996).

The results from all bioassays were analyzed using ToxCalc Version 5.0 (Dunnett's ANOVA, $\alpha=0.05$). The results from the water and sediment bioassays were then compared to that of ExxonMobil and Arcadis' analytical data for aqueous and sediment samples most closely corresponding with sampling sites in this study (Figure 1, Table 4).

Results

Neither survival nor growths were significantly different from controls in *P. promelas* for either sampling date at any sampling sites. However, a significant decrease in *C. dubia* reproduction was measured in water collected in June at the cove site (Site 4). Additionally, significant decreases in *C. dilutus* growth were measured in sediment collected at Site 2 and 4 (June collection) and site 2 and 5 (September collection) (Table 1). ASU did not measure the chemical composition of collected water and sediment samples, however, the toxicity measurements obtained through WET testing was compared to analytical measurements done by ExxonMobil and Arcadis. Therefore, Table 1 includes the toxicity measurements from bioassays performed by ASU, while Tables 2 and 3 include the chemical composition data for sediment and water published by ADEQ that is comparable to the toxicity measurements performed in this study for the corresponding sites.

Table 1. Results from aqueous and sediment toxicity tests; *C. dubia* and *P. promelas* were exposed to water samples while *C. dilutus* were exposed to sediment both collected in June and September 2013. Endpoints include: % survival and reproduction (+/-SD) for *C. dubia* and % survival and growth (+/-SD) for *P. promelas* and *C. dilutus*. *=indicates significant difference from the control at $\alpha=0.05$.

ToxCalc Results from Aquatic Organisms Exposed to Mayflower Water/Sediment						
Sampling Sites	<i>C. dubia</i> (June 2013)		<i>P. promelas</i> (June 2013)		<i>C. dilutus</i> (July 2013)	
	Survival (%)	Reproduction	Survival (%)	Growth (mg)	Survival (%)	Growth (mg)
Site 1	100±0.0	33.7±3.97	98±0.06	0.20±0.01	70±0.25	2.88±1.01
Site 2	100±0.0	32.3±3.47	98±0.06	0.21±0.02	95±0.09	2.18±0.20*
Site 3	100±0.0	30.6±1.58	100±0.0	0.22±0.01	87±0.14	2.10±0.31
Site 4	100±0.0	22.4±4.74*	100±0.0	0.18±0.02	78±0.23	1.89±0.49*
Site 5	100±0.0	29±4.24	98±0.06	0.21±0.01	80±0.15	2.18±0.24
Site 6	100±0.0	29±3.18	93±0.10	0.21±0.02	86±0.14	3.07±0.76

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Table 1 cont'd

Sampling Sites	<i>C. dubia</i> (Sept. 2013)		<i>P. promelas</i> (Sept. 2013)		<i>C. dilutus</i> (Sept. 2013)	
	Survival (%)	Reproduction	Survival (%)	Growth (mg)	Survival (%)	Growth (mg)
Site 1	80±0.19	25.6±8.80	98±0.06	0.41±0.05	80±0.16	2.82±0.5
Site 2	100±0.0	24.1±8.31	95±0.07	0.40±0.02	95±0.12	2.25±0.38*
Site 3	100±0.0	25.5±4.95	100±0.0	0.38±0.02	78±0.22	2.69±0.48
Site 4	90±0.15	24.9±5.95	98±0.06	0.37±0.01	87±0.16	2.97±0.40
Site 5	no water	no water	no water	no water	58±0.28	2.04±0.34*
Site 6	100±0.0	16.1±3.14	95±0.07	0.38±0.05	57±0.30	3.13±0.48

Table 2. Selected chemicals in water samples collected by ExxonMobil/Arcadis (Figure 1C). N.D. = chemical was not detected; J = compound was positively identified, however, the associated numerical value is an estimated concentration only.

ExxonMobil, Arcadis Mayflower/Lake Conway Water Sampling Results					
Sites	7	8	10	12	
Date	6/7/2013	9/24/2013	6/7/2013	9/24/2013	9/24/2013
Depth (m)	0.15-0.31	0.15-0.31	Surface	0.46-0.61	0.46-0.61
Acetone (µg/l)	4.3 ^J	7.4	3.3 ^J	3.5 ^J	N.D.
Benzo(a)anthracene (µg/l)	0.22	N.D.	N.D.	N.D.	N.D.
Benzo(a)pyrene (µg/l)	0.22	N.D.	N.D.	N.D.	N.D.
Pyrene (µg/l)	0.65	N.D.	0.011 ^J	N.D.	N.D.
Arsenic (mg/l)	0.0108 ^J	N.D.	N.D.	N.D.	N.D.
Chromium (mg/l)	0.0167	N.D.	0.0024 ^J	N.D.	N.D.
Lead (mg/l)	0.0306	N.D.	N.D.	N.D.	N.D.
Vanadium (mg/l)	0.0243	N.D.	0.0024 ^J	N.D.	N.D.

Table 3. Selected chemicals in sediment samples of Lake Conway/Mayflower collected by ExxonMobil & Arcadis (Figure 1A) correspond to those in this study; dates above are the only available for sediment samples. *Site 16, 17 (Lake Conway cove) and 37, 38 (Lake Conway) were averaged since each were close to site 4 and 2 respectively. All were collected at 0-0.15 m. J = compound was positively identified; however, the associated numerical value is an estimated concentration only. TPH = Total Petroleum Hydrocarbons.

ExxonMobil & Arcadis Lake Conway/Mayflower Sediment Samples						
Sites (Figure 1)	4	9	16, 17	34	37, 38	
Dates	8/15/2013	7/31/2013	8/2/2013	8/5/2013	7/27/2013	7/28/2013
Acetone (µg/kg)	62 ^J	33 ^J	23	40.5	78	120
Benzo(a)anthracene(µg/kg)	29.8	9.46	0.33	8.7	17	34.3
Benzo(a)pyrene (µg/kg)	47.9	10.9	0.14	29	14.4	30.9
Pyrene (µg/kg)	131	0.745	24.5	51.9	33.6	68.5
Arsenic (mg/kg)	11.5 ^J	3.6	4.6	4.5	5.8	8.8
Chromium (mg/kg)	21.2 ^J	18.0 ^J	13.3	17.55	28.4	30.5 ^J
Lead (mg/kg)	18.7	18	12	19	40.8	38.8 ^J
Vanadium (mg/kg)	23.1 ^J	23.4	21.6	28.1	46.7	48.8
TPH (mg/kg)	2277	300	51	995.5	558	689

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Discussion

The toxicity that was measured in this study for sediment showed a decrease in growth for *C. dilutus* in sites 2 and 4 in June and sites 2 and 5 in September, while toxicity was measured in the water from site 4 in June with a decrease in reproduction for *C. dubia* (Table 1). Site 2 was located inside the body of Lake Conway where there was reportedly no oil contamination. However, sites 4 and 5 were closer to the location of the spill. Site 4 was located in the cove of Lake Conway which was contaminated by the spill. Site 5 was close to the housing division in which the pipeline burst, located in a drainage ditch under railroad tracks.

All of the chosen chemicals tested by ExxonMobil and Arcadis were detected for each of the corresponding sites measured for toxicity in this study (Table 2). For example, the levels of TPH in sediment were the greatest for the sites sampled in this study in which toxicity was measured. While lower than the TPH levels, the level of the three different types of PAHs in these three sites sediment sites were also the greatest. According to the results compiled by ADEQ, the levels of PAHs and TPHs from the spill were great enough to cause the toxicity measured at these sites in our study.

Summary of Water Sample Data

As previously mentioned, WET testing toxicity in this study was only measured in site 4 or the cove of Lake Conway, showing a decrease in the reproduction of *C. dubia* as compared to the control. When this was compared to the results from ExxonMobil and Arcadis for this specific date in the corresponding cove site, it can be seen that all of the selected chemicals were detected. This correlation could possibly be a reason for the toxicity measured in the aqueous samples from site 4 (Table 2). When either *D. Magna* or *D. pulex* (as discussed in above introduction) were exposed to these chemicals in toxicity testing, the endpoints were greater for each of the chemicals than the measured value by ExxonMobil and ADEQ (Table 2). For example, the toxic endpoint of *D. pulex* when exposed to benzo(a)anthracene is greater than the measured value (LC50 10 µg/L, *D. pulex*; measured value 0.22 µg/L) (Table 2). Since *D. pulex* are larger than *C. dubia* used in this study, they are more tolerant and thus the smaller, more sensitive *C. dubia* will be sensitive to levels measured at this site (Bossuyt and Janssen 2004). While the site in the cove that was sampled by ExxonMobil and Arcadis was closer to the

main body of the lake and was not in the same exact location of the site measured in this study, it can be inferred that if these chemicals were detected farther away from the point of the spill they might possibly of higher concentration closer to point of the spill, such as the location of site 4 (Figure 1).

Summary of Sediment Sample Data

The toxic results measured from the sediment were possibly due to the increased levels of TPHs and PAHs found in corresponding ADEQ sites. Toxicity was measured in the sediment from sites 2 and 4 for the June sampling date; *C. dilutus* exposed to this sediment showed a decrease in growth as compared to the control sediment used. *C. dilutus* exposed to sediment from sites 2 and 5 for the September sampling date also showed a decrease in growth. When compared to the data from ExxonMobil and Arcadis sites 37/38, 16/17, and 4 (corresponding respectively with sites 2, 4 and 5 from this study) have the greatest measured TPH levels as well as considerable PAHs such as benzo(a)anthracene, benzo(a)pyrene, and pyrene (Table 2). Therefore, there was a consistent sublethal effect detected in site 2 between the two sampling dates, as well as toxicity measured in sites 4 and 5 which were in close proximity to each other.

Even though the sampling dates were not the same as those done by ExxonMobil and Arcadis, it can be inferred that the chemicals from oil spills leach, as they remain in the sediment for extended periods especially concerning this type of crude oil. For example, approximately four million liters of heavy crude oil or dilbit leaked into the Kalamazoo River in 2010 and remnants still remain in the floodplains, riverbanks and sediment of the river (Brooks 2014). An Environmental Working Group study on the Mayflower oil spill states that chemicals from crude oil, especially dilbit can remain in sediment for at least three years as this is when it was determined the Kalamazoo River would need to be dredged (Sharp et al. 2013). Therefore the settling of heavy chemicals from the crude oil is most likely the reason for the measured sediment toxicity at those sites.

Conclusions

Toxicity was measured in organisms exposed to water and sediment contaminated by oil. There were three sites in which toxicity was measured, one was very close to the point of the spill (site 5 drainage ditch), the other in the cove of Lake Conway (as far as oil reportedly reached) and lastly inside the main body

of the lake. Water and sediment toxicity was measured in *C. dubia* (reduced reproduction) and *C. dilutus* (reduced growth), however, *P. promelas* showed neither a decrease in survival or growth. Previous research has shown that invertebrates, such as *C. dubia*, are more sensitive to contaminants than vertebrates (Bossuyt and Janssen, 2004). Also, the prediction that was made stating that the sites close to the point of the oil spill would be more likely to measure toxicity was also inconclusive. While toxicity was measured in site 4 (*C. dubia*) and sites 4 and 5 (*C. dilutus*) which were close to the point of the spill, there was also toxicity measured in site 2 (*C. dilutus*) inside the lake, perhaps due to the natural flow of the water and the accumulation of heavier constituents of the crude oil into the sediment.

Even though daily water samples were taken, sediment sampling done by ExxonMobil and Arcadis did not begin until July 27, 2013 (ADEQ 2013). The toxicity in sediment from site 2 was measured although the oil reportedly did not reach the main body of the lake (Figure 1A-B). Exxon deployed 1097 m (3600 ft) of containment (or hard) booms between point of the oil spill and Lake Conway (Duke 2013). This type of boom not only contains buoyant material that keeps it afloat and prevents oil from leaking, but also contains a skirt below the surface extending to the bottom which is designed to prevent oil from escaping underneath (NOAA Office of Response and Restoration 2015). This prevents the oil from spreading and provides easy removal. It is interesting that toxic results as well as measured constituents of the crude oil were measured at this site, indicating that the boom was not completely effective in preventing movement of contaminants into the lake. The water and sediment toxicity results can be compared and correlated between the two sampling dates in that there was no toxicity measured for each in site 4 for September. However, there is no analytical data to compare the sediment toxicity results of the September sampling date in site 5. The reduced growth of *C. dilutus* for site 5 in September indicates some residual chemical remained several months after the spill. This is of concern due to the proximity to the neighborhood and the recalcitrant nature of some of the chemicals present in the crude oil.

The analysis reported in this experiment on the effects of the Mayflower oil spill highlight the importance of preventing similar occurrences from happening. Oil spills, especially those occurring from underground pipelines carrying heavy crude oil can cause extensive damage. The Mayflower oil spill

leaked a significant portion of heavy crude into the surrounding neighborhood, reaching the cove of Lake Conway. This not only affected the individuals living in the neighborhood, but also disturbed the water running into storm drains and ditches, ultimately leading to the cove of Lake Conway. The type of oil in the Pegasus pipeline in Mayflower contained many contaminants that are toxic to aquatic organisms. Appropriate maintenance of the pipeline infrastructure could have protected the individuals living close to the pipelines as well as Lake Conway. Maintenance of pipelines can prevent leaks especially if the pipelines are going to be carrying heavy crude, such as dilbit, which causes pressure swings, damaging old pipelines. Although Exxon Mobile was mostly effective in cleaning up the initial spill and the containment booms prevented most of the oil from reaching the main body of the lake, toxic effects were visible. The toxicity measured in aqueous organisms inside the lake at site 2 confirmed some contaminant movement beyond the booms. Continual sampling of the affected area, including the use of bioassays would increase the understanding of post-spill effects on the environment.

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Natural Nutrient Sources in the Cache River Watershed, Arkansas

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Running Title: Natural Sources of Nutrients

Abstract

The growth of the hypoxic ‘dead zone’ in the Gulf of Mexico in recent years has placed increased focus on potential sources of nutrient pollution, with most of the focus being placed on watersheds where practices, including fertilizer application and land alterations combine to increase non-point source runoff. In this study, nutrient concentrations in surface waters of altered and unaltered areas of the Cache River Watershed, Arkansas, were compared to determine if agricultural land usage was responsible for the majority of nutrient inputs. Results suggest that for dissolved nitrites and orthophosphates, agricultural (altered) sites contribute significantly more than relatively unaltered sites but that for dissolved nitrates, unaltered sites have a large contribution to overall nitrate concentrations, particularly in late summer and fall months.

Introduction

In recent years, the growth of the so-called ‘dead zone’ in the Gulf of Mexico has placed increased focus on potential causes of this area of hypoxia (Malakoff 1998, Dodds 2006). The primary source of contamination is thought to be nutrient pollution, specifically nitrogen and phosphorus inputs, from the Mississippi River Basin (Rabelais et al. 2002). This watershed drains approximately 41% of the continental United States, including some of the most agriculturally productive regions, in the central and mid-western United States (USEPA 2014).

Agricultural production in the United States accounts for roughly 21% of the overall worldwide production (approximately 1013.37 million metric tons) with major contributions from corn, coarse grains, wheat, soybeans, oilseed and cotton (USDA 2013). Between 1960 and 2011, production of crops more than doubled while fertilizer application nearly tripled (USDA 2013). These fertilizers, along with agricultural land usage practices, are thought to be the

primary contributors to the hypoxic zone in the Gulf of Mexico (White et al. 2014)

In the United States, Arkansas is a major contributor to overall crop production, ranking 12th nationwide (USDA ERS 2013), including ranking 1st in rice production, and 5th in cotton production (USDA ERS 2013). The Delta Ecoregion of northeastern Arkansas is the major region of agricultural production in this state, with agricultural land usage ranging between 50 and 80% by watershed (AWIS 2006).

Due to this intense agricultural usage, several watersheds in northeastern Arkansas have been named as focus area watersheds by the Mississippi River Basin Initiative (MRBI) in the attempt to limit the influence on the hypoxic zone in the Gulf of Mexico (USDA NRCS 2012). While the majority of these watersheds have been heavily altered for agricultural production, some relatively unaltered areas remain.

In the Cache River Watershed, the presence of a unique geological feature, Crowley’s Ridge, along the eastern side of the watershed, has resulted in a portion of the watershed being left relatively unaltered, due to its unsuitability for traditional row-cropping. Forest cover in these portions of the watershed are as high as 65% of land area compared to less than 10% in agriculturally productive areas of the watershed (AWIS 2006). This watershed, headwatered in southeastern Missouri, accounts for approximately 12.1% of the land area in the Delta ecoregion (Scott et al. 1998). Because of the importance of this watershed as an agriculturally valuable resource as well as a potential source of nutrient contamination leading to the hypoxic zone, it has been identified as a focus area watershed by the MRBI and a watershed of concern by the Arkansas Department of Environmental Quality (ADEQ) (ADEQ 2012).

Here we examine nutrient concentrations in mixed-use sub-watersheds of the Cache River Watershed over two growing seasons (2013-2014). Seven unaltered and three altered sites were examined, ranging across five sub-watersheds of the Cache River. Altered sites were

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characterized by artificial stream channelization, removal of riparian vegetation and conversion of surrounding land to agricultural usage. Unaltered sites retained natural stream contours, a riparian buffer and overhanging canopy and were primarily surrounded by forested or pasture land.

Based on characteristics of altered sites, namely the lack of stream contours and riparian vegetation, it was predicted that runoff due to precipitation would be greater at these sites than at unaltered sites. Combined with the increased use of artificial fertilizers on land surrounding these sites, this was expected to lead to increased nutrient levels at altered sites when compared to unaltered sites. This difference was predicted to be greatest following heavy precipitation events, when nutrient-containing runoff should be greatest.

Materials and Methods

Site selection and sampling frequency

Seven unaltered and three altered sites were selected within five sub-watersheds of the Cache River. Channel widths and depths were relatively constant for all sites sampled, with widths less than 10 m and depths ranging from 0.2 m to 1.5 m, depending on precipitation. Samples were collected bi-monthly over the length of the agricultural season (May–October) for two years, 2013 and 2014.

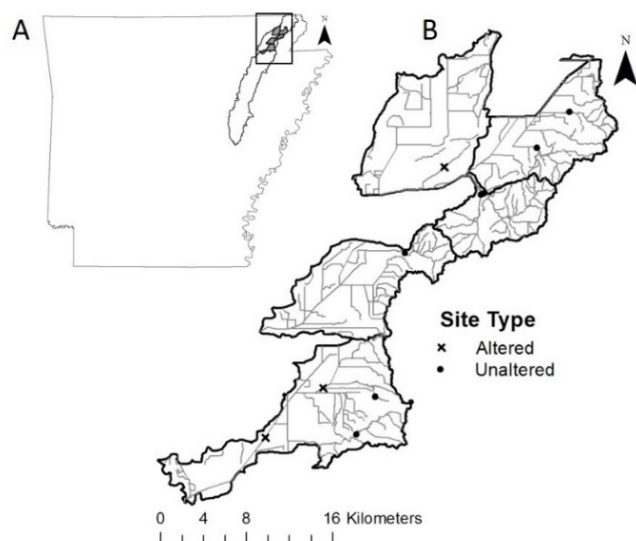


Figure 1. A) Location of Cache River watershed in Arkansas and sampled sub-watersheds within Cache River watershed (in box). B) Location of sites sampled within five sub-watersheds of the Cache River.

Sample analysis

Water samples were collected from vertical centroid of the water column and immediately analyzed for temperature, dissolved oxygen (DO), conductivity and pH using an Orion Star A329 multiprobe field meter (Thermo Scientific). Collected water was placed in an acid-washed Nalgene container and stored at 4°C until analyzed. For each sampling year, one 10 mL sample was filtered using a 0.45 µm filter (Environmental Express) for analysis of dissolved nutrients (nitrate (NO_3^-), nitrite (NO_2^-), orthophosphate(PO_4^{3-})) using either a discrete nutrient analyzer (DA 3500, OI Analytical) or a flow-through analyzer (Skalar San++). For year two, an additional 40 mL of unfiltered water was collected and transported to the Arkansas State University Ecotoxicology Research Facility (ASU ERF) for digestion (APHA method 4500- NO_3F and 4500-P (APHA 2005)) and analysis of total nitrogen and total phosphorus (Skalar San++). At this time, nutrient criteria for this region have not been set by the state of Arkansas. Therefore, 25 percentile values for total nitrogen and total phosphorus were compared to recommended nutrient criteria for the ecoregion as proposed by the United States Environmental Protection Agency (USEPA 2001).

Statistical analysis was performed using R and R Studio (R Core Team 2015). All data sets were tested for normality using a Shapiro-Wilks test and transformations were applied when data were not normally distributed. If transformations failed to achieve normality, non-parametric statistical tests were employed.

Results

Dissolved nutrient data was not normally distributed and transformations failed to achieve normality. Thus, non-parametric statistical tests were used for comparisons. Dissolved NO_2^- and PO_4^{3-} values were significantly lower at unaltered sites than at altered sites (Mann-Whitney U-test, $p < 0.001$ (NO_2^-), $p < 0.001$ (PO_4^{3-})). No significant difference was detected between site types for dissolved NO_3^- (Mann-Whitney U-test, $p = 0.17$), though levels were greater at unaltered sites than altered sites.

NO_3^- concentrations at altered sites were correlated with precipitation (Spearman's rank correlation, $p = 0.03$) with spikes in NO_3^- typically occurring 24–48 hours after heavy rainfall. No correlation was found between NO_2^- or PO_4^{3-} at altered sites or between any dissolved nutrients and precipitation at unaltered sites.

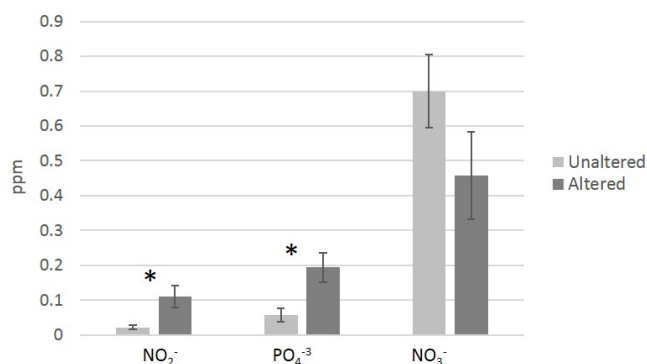


Figure 2. Comparison of dissolved nutrient (mean \pm S.E.) concentrations at altered (n=3) and unaltered sites (n=7). Asterisk denotes a significant difference between mean concentrations at site types ($\alpha=0.05$).

Comparing total nutrient concentrations between site types showed a similar statistical pattern. Total phosphorus was significantly greater at altered sites than at unaltered sites (t-test for unequal variance, $t = -2.434$, $p=0.03$) while no significant difference was detected between total nitrogen based on site type (t-test for unequal variance, $t=-1.911$, $p=0.076$).

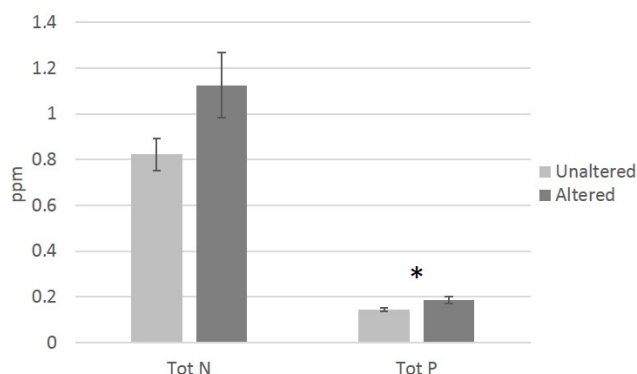


Figure 3. Comparison of total nitrogen and total phosphorus (mean \pm S.E.) concentrations at altered (n=3) and unaltered sites (n=7). Asterisk denotes a significant difference between mean concentrations at site types ($\alpha=0.05$).

Discussion

In highly managed agroecosystems, fertilizer application, combined with altered landscape features, may often result in increased loss of nutrients to receiving waterways (Carpenter et al. 1998, Sims et al. 1998), especially when compared to relatively unaltered areas (Wang et al. 2014). In the Cache River Watershed, we measured this pattern to hold true for our sampling period, with significantly higher concentrations of dissolved PO₄⁻³ and NO₂⁻ in waterways surrounded by altered, agriculturally

productive land than in those with less altered, forested watersheds. However, we found decreased concentrations of NO₃⁻ at altered sites, compared to unaltered sites.

Nutrient criteria are typically described in terms of qualitative data rather than quantitative. As such, no numeric criteria have been set by the state of Arkansas at this time for this ecoregion. However, the USEPA has proposed nutrient criteria for total nitrogen and total phosphorus based on a larger ecoregion (Ecoregion X) composed of the Texas/Louisiana coastal plains and Mississippi Alluvial plains. This criteria is based on the 25th percentile. Accordingly 25th percentile values were calculated for total nitrogen and total phosphorus for both altered and unaltered sites. In all cases 25th percentile values fell below or just slightly above proposed criteria limits, indicating that the levels detected are probably not of immediate environmental concern. During the course of this study, site observations did not indicate any qualitative indicators of enrichment, such as algal blooms.

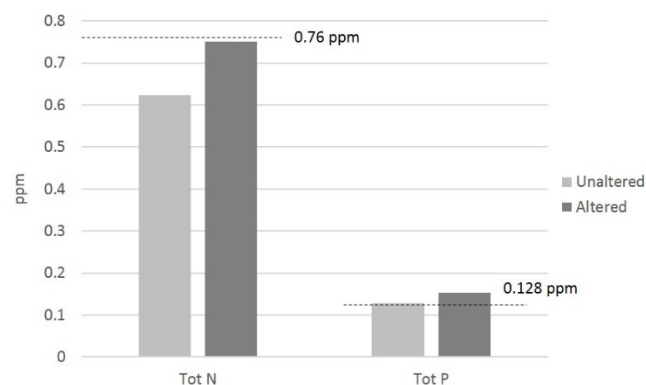


Figure 4. Comparison of total nitrogen and total phosphorus (25th percentile) concentrations at altered (n=3) and unaltered sites (n=7) to USEPA proposed criteria for Ecoregion X (dashed lines).

The decrease in NO₃⁻ at altered sites occurs in late summer/early fall and matches the time when maximum nutrient uptake by crops would be expected to occur (University of Arkansas, Cooperative Extension Service 2015). Earlier in the growing season, losses to waterways were greater, most likely due to lower uptake by crops and greater precipitation. A significant correlation did exist for precipitation in the 48-hr period before sampling and NO₃⁻ concentrations in waterways surrounded by altered landscapes.

More puzzling is the increased concentrations of NO₃⁻ in waterways surrounded by unaltered landscapes

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in summer/fall months. Typically, NO_3^- concentrations follow a seasonal pattern with a summer minima and a late fall/winter maxima (Jayasinghe et al. 2012). However, we saw NO_3^- levels begin to increase in summer (August 2014, July 2013). While in the beginning of the growing season, NO_3^- concentrations were greater at altered sites than unaltered sites, this relationship reversed for the latter half of the season. This increase was largely attributable to values from three of the seven natural sites but was not correlated with precipitation in the 72-hr period preceding sampling, indicating it was most likely not a result of surface runoff.

The increase in NO_3^- at unaltered sites is most interesting as these sites typically display better water quality (lower concentrations of potential pollutants) than altered sites. An examination of NO_3^- concentrations in agricultural watersheds using the SWAT model indicates that NO_3^- concentrations are generally positively correlated with acres of land used for row cropping (summarized in Jayasinghe et al. 2012).

Several possible explanations exist to explain the increase in NO_3^- at unaltered sites. Firstly, it is possible that an unrecognized artificial source of nutrients exists in these watersheds. While crop-based agriculture is not prevalent, some animal-based agriculture does exist, primarily pastured cattle. Nutrient outputs from animal agriculture have been recognized as a source of nutrient impairments in other Midwestern watersheds (Keeney and DeLuca 1993). Nitrogen leaching from grazed pastures has been found to be similar in amount to nitrogen leached from row-cropped areas, largely due to the urination of animals (Di and Cameron 2002). Because increased NO_3^- concentrations were only noted in three of the seven unaltered creeks, a further examination of these sites is in order.

Secondly, the increase could represent a combination of increased soil nitrogen and decreased plant uptake. Unaltered sites have much more forest cover and thus more vegetative litter. As this litter falls to the ground, its decomposition by nitrifying organisms leads to increases in soil reservoirs of NO_3^- . These reservoirs would typically be depleted by plant growth. However, later in the growing season, plant uptake of nutrients would decrease, leaving more to wash into surface waters either as runoff or via subsurface flows. Bechtold et al. (2003) found that subsurface inputs of water were enriched in NO_3^- and took longer to reach surface channels than surface runoff.

An examination of precipitation data indicates no significant correlation between in-stream NO_3^- levels at unaltered sites and precipitation totals for the 72 hrs prior to sampling. This indicates that surface runoff of NO_3^- is not the primary contributor to stream NO_3^- levels. Extending this analysis to precipitation totals for one week and two weeks prior to sampling also reveal no significant correlation, indicating that subsurface flow is also unlikely to be the primary contributor to elevated surface water concentrations. It is important to note that because this is a highly agricultural area, irrigation during dry seasons is common. Thus, precipitation totals alone might not account for all water inputs to a stream. The influx of irrigation water at altered sites could potentially dilute surface water concentrations of dissolved NO_3^- . Because unaltered sites would not receive similar amounts of irrigation water, such dilution would not occur at these sites, making them appear to have elevated NO_3^- concentrations when compared to altered sites.

A final possibility for increased surface water concentrations at unaltered sites is due to nitrifying organisms in the stream, which would convert substrate or particulate bound nitrogen into NO_3^- . While altered stream sites could have similar nitrification rates, these streams are less likely to retain nitrogen, due to a decrease in habitat heterogeneity as a result of channelization and removal of riparian vegetation (Kemp and Dodds 2002). This is supported by our observation of similar amounts of total nitrogen in altered and unaltered streams, but dissimilar amounts of dissolved NO_3^- .

This hypothesis is also supported by a negative (though only marginally significant ($p=0.11$)) relationship between precipitation and NO_3^- concentrations at unaltered sites. When precipitation is greater, NO_3^- concentrations are lower (indicating dilution) and vice-versa. The same dilution could occur during times of low precipitation at altered sites when irrigation would add water to the system, thus explaining the low NO_3^- concentrations at times of low precipitation at altered sites. A similar inverse relationship between flow and NO_3^- concentrations was described in headwater streams in the Northeastern United States, though the streams in question had a seasonal snowpack, thought to contribute to overall NO_3^- (Goodale et al 2009). These authors also noted a seasonal pattern similar to what we observed, with peaks in NO_3^- in summer months, during the peak of the growing season.

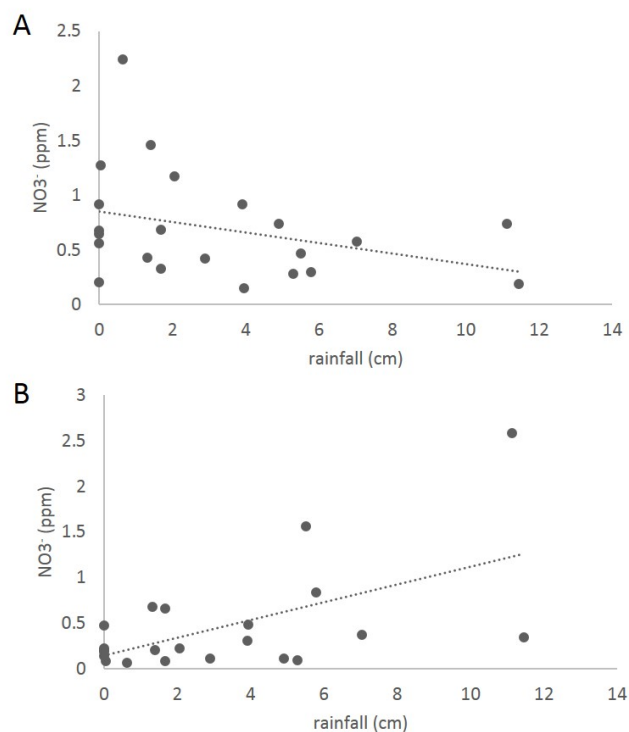


Figure 5. A) Relationship between NO_3^- concentrations and total precipitation for one week prior to sampling for unaltered sites B) Relationship between NO_3^- concentrations and total precipitation for one week prior to sampling for altered sites.

Conclusions

Differences in dissolved NO_2^- and PO_4^{3-} between site types were as expected. However, the increase in dissolved NO_3^- observed at unaltered sites indicates that an unidentified, and possibly natural, source of nutrients exists. Future testing should include an examination of other potential sources of nitrogen, including potential contributions from vegetation, subsurface flows, in-stream nitrification or unidentified anthropogenic sources. Also, extending sampling throughout the year, rather than just during the growing season, would provide greater insight into typical annual fluctuations in nutrients at these sites. Concentrations of total nitrogen and phosphorus were below or very similar to proposed nutrient criteria for the ecoregion, indicating that levels measured are most likely not of immediate environmental concern.

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Coccidia (Apicomplexa: Eimeriidae) of Three-toed Box Turtles, *Terrapene carolina triunguis* (Reptilia: Testudines), from Arkansas and Oklahoma

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Running Title: Coccidia of Turtles

Abstract

We collected 50 three-toed box turtles (*Terrapene carolina triunguis*) from 9 counties of Arkansas and 4 counties of Oklahoma, and examined their feces for coccidial parasites. Nine of 24 (38%) turtles from Arkansas and 8 of 26 (31%) from Oklahoma were found to be passing oocysts of *Eimeria ornata*. This represents two new geographic distributional records for this coccidian. Measurements of individual isolates of *E. ornata* as well as morphological characteristics are provided with comparison to its original description and to another *Terrapene* coccidian, *Eimeria carri*. In addition, we noted an adelid pseudoparasite being passed by a single *T. c. triunguis* from Oklahoma that likely represents a parasite of arthropods.

Introduction

Much has been written on the natural history and ecology of North American box turtles, *Terrapene* spp. (Dodd 2001). There is also a great deal of information available on their endoparasites (Ernst and Ernst 1977, and others). However, little is known about coccidian parasites of box turtles. McAllister and Upton (1989a) were the first to summarize the coccidians (Apicomplexa) of turtles and, more recently, Duszynski and Morrow (2014) provided a summation on the coccidia of turtles of the world. In the genus *Terrapene* Merrem only 3 species of coccidia have been described and/or reported as follows: *Eimeria carri* Ernst and Forrester originally reported from the eastern box turtle, *Terrapene carolina carolina* from Alabama and Florida (Ernst and Forrester 1973) and later found in three-toed box turtles, *Terrapene carolina triunguis* from Arkansas (McAllister et al. 1994); the second coccidian is *Eimeria ornata* McAllister and Upton reported from *Terrapene ornata*

ornata from Texas (McAllister and Upton 1989b); and the third is the ubiquitous *Eimeria mitraria* (Laveran and Mesnil) Široký, Kamler and Modrý, reported from *T. c. triunguis* from Arkansas (McAllister et al. 1994). To date, as far as we know, neither of the first two species has been reported from additional turtles or additional US states. Here, we report, for the first time, *E. ornata* from *T. c. triunguis* from Arkansas and Oklahoma, and provide comparative measurements of individual isolates as well as data on their morphological characteristics.

Materials and Methods

Between March 2012 and May 2015, we collected 50 juvenile and adult *T. c. triunguis* by hand or as salvaged road-killed (DOR) specimens in nine (Benton [*n*=1], Boone [*n*=1], Fulton [*n*=1], Little River [*n*=2], Marion [*n*=2], Montgomery [*n*=1], Pike [*n*=4], Pope [*n*=1], Union [*n*=11]) counties of Arkansas and four (Latimer [*n*=4], Le Flore [*n*=3], McCurtain [*n*=18], Pushmataha [*n*=1]) counties of Oklahoma. In the laboratory, live turtles were held in 38L glass terrariums and, immediately after defecation, each fecal sample was placed in a vial containing 2.5% (w/v) aqueous potassium dichromate solution (K₂Cr₂O₇); these turtles were released back into the wild. Feces were obtained from DOR turtles by taking samples directly from the rectum. Following an initial examination, all positive samples were transferred to Petri dishes containing a thin layer of K₂Cr₂O₇ and allowed to sporulate completely for up to 1 wk. Following sporulation, oocysts were concentrated by flotation in a modified Sheather's sugar solution (sp. gr. 1.30) and examined using light microscopy, photographed with Nomarski interference-contrast optics, and measured with a calibrated ocular micrometer or Olympus® cellSens 1.7 digital software.

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Measurements are reported in micrometers (μm) with means followed by the ranges in parentheses. Descriptions of oocysts and sporocysts follow guidelines of Wilber et al. (1998) as follows: oocyst length (L) and width (W), their ranges and ratios (L/W), micropyle (M), oocyst residuum (OR), polar granule(s) (PG), sporocyst length (L) and width (W), their ranges and ratio (L/W), sporocyst (SP), Stieda body (SB), substieda body (SSB), parastieda body (PSB), sporocyst residuum (SR), sporozoites (SZ) anterior (ARB) and posterior (PRB) refractile bodies, and nucleus (N). A host photovoucher was accessioned into the Arkansas State University Museum of Zoology, Herpetology Collection (ASUMZ), State University, AR as ASUMZ 32041. Photovouchers of sporulated oocysts were accessioned into the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, NE as HWML 101825-101827.

Results

Nine of 24 (38%) three-toed box turtles from Arkansas and 8 of 26 (31%) from Oklahoma were found to be passing oocysts of *E. ornata* (Fig. 1); overall prevalence was 17 of 50 (34%).

A description of the oocysts ($n = 89$) we observed are as follows: sporulated oocyst with four sporocysts; shape spheroidal to subspheroidal; smooth uni-layered wall, colourless, ~ 0.5 thick; $L \times W$: 18.2×16.0 ($17-19 \times 15-17$); L/W : 1.1 (1.1–1.2); M absent, OR present as compact granulated spheroidal mass frequently surrounded by membranous sac, PG (1-2) present. SP ellipsoidal to elongate, smooth uni-layered wall; $L \times W$: 11.3×5.2 ($11-12 \times 5.1-5.4$); L/W : 2.2 (2.0–2.3); SB present as a distinct point on end of SP, SSB, PSB absent; SR: composed of small granules dispersed throughout; SZ: (not measured) sausage-shaped, lying lengthwise in SP with large and distinct subspheroidal ARB and PRB; N slightly off center of SZ.

We also found one of 26 (4%) turtles from Oklahoma to be passing oocysts of an unknown genus of coccidian with oocysts containing many (>12) sporocysts (Fig. 2). We consider this an adelid pseudoparasite that was likely ingested by this turtle with its arthropod host.

Discussion

Herein, we have provided the largest survey, to date, on coccidia of *T. c. triunguis*. In comparing our samples of *E. ornata* to those originally described by McAllister and Upton (1989b) from a different host (*T.*

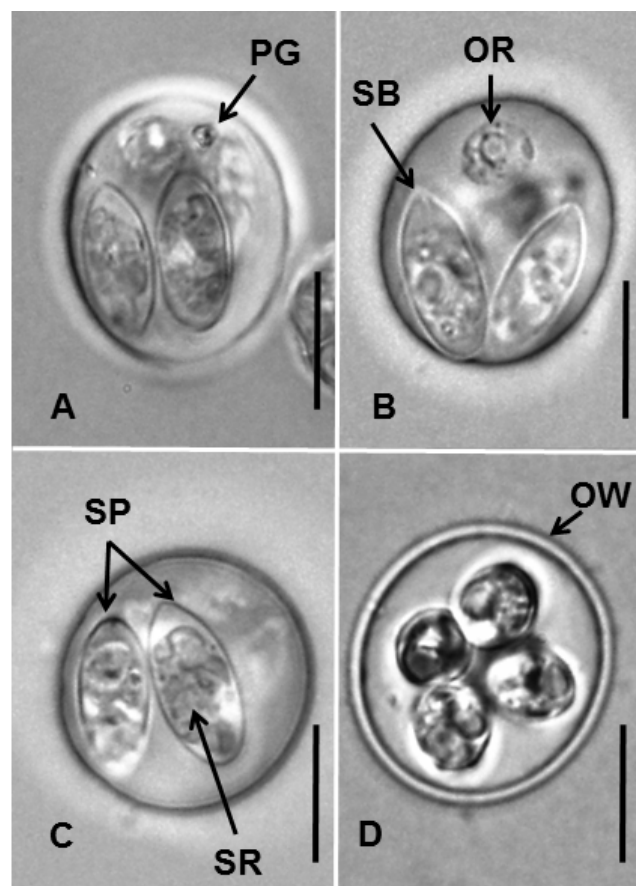


Figure 1. Sporulated oocysts of *Eimeria ornata*. A. Oocyst from isolate Tct1 showing polar granule (PG); B. Oocyst from isolate Tct6 showing oocyst residuum (OR) and point on Stieda body (SB); C. Oocyst from Tct6 showing typical elongate shape of sporocysts (SP) and sporocyst residuum (SR); 4. Oocyst from isolate Tct33 showing single layered oocyst wall (OW). Scale bars = 10 μm .

o. ornata) and locality (Texas) we observed the following: oocyst and SP shape and size ($17.9 \times 15.7 \mu\text{m}$, 11.1×5.4 in original description) were similar as well as L/W ratios, and SR; differences were observed in the appearance of the OR as we did not see a vacuole in the middle but closer to the edge, and the oocyst wall was listed originally as being thicker at 1.0 μm . However, these differences are minor and could be the result of using different microscopic optics.

We did observe some differences in sizes of oocysts and SP between the 6 isolates that were measured (Table 1). However, these differences are not considered significant and we are confident that all isolates represent *E. ornata*.

Duszynski and Morrow (2014) argue that *E. carri* and *E. ornata* may represent the same species. We disagree because there are enough differences in oocysts between the two, particularly in the fact that *E. carri* has never been reported to have polar granules,



Figure 2. Unknown adelid coccidian containing many sporocysts from *Terrapene carolina triunguis* from Oklahoma. Scale bar = 10 μ m.

but *E. ornata* often does, and oocysts are smaller in *E. carri* by at least 2.0 μ m and average $15.9 \times 14.5 \mu$ m. In addition, there are also some minor structural differences, marked differences in sporulation, and no photomicrograph of *E. carri* exists. Ideally, molecular confirmation would be needed to resolve this question.

In conclusion, we have provided additional information on the turtle coccidian, *E. ornata*. We document 2 new geographic distribution records for this coccidian. Although there is information on coccidia of other turtles in Arkansas, including *Chelydra serpentina* (McAllister et al. 1990), *Macrochelys temminckii* (Upton et al. 1992) and various emydid, kinosternid, and trionychid turtles (McAllister et al. 1994), additional surveys are recommended on other turtles of the state and, especially, those from Oklahoma.

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Table 1. Comparative data on isolates of *Eimeria ornata* from *Terrapene carolina triunguis* (Tct). Eighty-nine oocysts were photographed, the mean for all oocysts measured ($n = 86$) $L \times W$ (μm) was 18.2×16.0 and the mean for all sporocysts measured ($n = 113$) $L \times W$ (μm) was 11.3×5.2 .

Isolate ¹⁻⁶	Oocysts Mean $L \times W$ (μm)	L/W	Sporocysts Mean $L \times W$ (μm)	L/W
Tct1-Pike Co., AR ($n = 5$) ¹	19.3×16.1 ($n = 4$)	1.2	11.4×5.3 ($n = 10$)	2.2
Tct2-Pike Co., AR ($n = 30$) ²	18.5×15.8 ($n = 29$)	1.2	11.0×5.4 ($n = 42$)	2.1
Tct6-McCurtain Co., OK ($n = 24$) ³	17.4×15.6 ($n = 24$)	1.1	11.4×5.1 ($n = 30$)	2.2
Tct21-Union Co., AR ($n = 5$) ⁴	16.9×15.4 ($n = 4$)	1.1	11.5×5.1 ($n = 4$)	2.2
Tct30-Fulton Co., AR ($n = 8$) ⁵	18.2×16.5 ($n = 8$)	1.1	10.9×5.1 ($n = 7$)	2.2
Tct33-McCurtain Co. OK ($n = 17$) ⁶	18.9×16.7 ($n = 17$)	1.1	11.5×5.1 ($n = 20$)	2.3

¹Little Missouri Bridge W of Daisy off US 70 (34° 14' 22.6428"N. 93° 50' 3.4944"W), collected 26 April 2012.

²vicinity of Kirby off US 70 (34° 15' 5.562"N, 93° 39' 29.559"W), collected 26 April 2012.

³Lukfata off Memorial Street (34° 00' 15.8394"N, 94° 45' 28.8108"W), collected 30 May 2013.

⁴Grady Bell Road, El Dorado (33° 12' 59.6514"N, 92° 35' 9.2142"W), collected 4 April 2012.

⁵Big Creek, SE of Mammoth Spring off US 63 (36° 26' 19.5282"N, 91° 29' 46.7082"W), collected 29 July 2013.

⁶Idabel off US 259 (33° 55' 19.473"N, 94° 46' 14.7714"W), collected 16 June 2013.

The Arkansas Endemic Flora and Fauna: An Update with 13 Additional Species

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Running Title: Arkansas Endemic Biota

Abstract

Arkansas supports a diverse variety of endemic biota with most found in the Interior Highlands (Ozarks and Ouachitas) of the state. Since 1988, several compilations have updated the number of endemics known while some former endemic species have been found in other states and subsequently removed from the state list. Here, update to the list by adding 13 taxa, several of which are fish parasites, making a grand total of 139 current endemic species in Arkansas.

Introduction

The first compilation of a list of endemic flora and fauna of Arkansas was provided by the Arkansas Department of Planning (1974) in which only 9 species were documented. Next, Robison and Smith (1982) listed 47 endemic taxa, Allen (1988) increased it to 85 species and Robison and Allen (1995) reported 117 endemic species. Due to recovery of some species in other states, Robison et al. (2008) reduced the list to 113 and McAllister et al. (2009) provided the most recent compilation of the endemic biota of Arkansas. That update brought the number of endemic species in the state to 126. Here, we update that list by adding 13 species, totaling 139 taxa, including 6 fish monogenean parasites the authors were not aware of during previous versions of this series.

List of Species-Material included. The following is a summary listing of the species added (Table 1).

Fungi: Basidiomycota: Agaricales: Physalacriaceae
Hymenopellis sinapicolor Peterson & Justice, 2010 in Peterson & Hughes, 2010 (a xeruloid mushroom).

This mushroom species was collected at Lake Sylvia Recreational Area, Saline County (Peterson and Hughes 2010). According to Drs. Petersen and Hughes, "ITS sequences from this taxon appear

identical to another species of *Hymenopellis*, *H. rugosoceps* (G.F. Atk.) R.H. Petersen. However, morphological characters are too disparate to allow synonymy. It is possible that basidiomata of *H. rugosoceps* are hypertrophied, and small spores are shared with *H. sinapicolor*. The two may represent different states of a single taxon." A more recent report of the species from Illinois has been posted on the world-wide web but this is not considered a refereed publication.

Plantae: Marchantiophyta: Jungermanniopsida: Fossombroniaceae

Fossombronia marshii Bray & Stotler (a liverwort).

Stotler et al. (2010) described this liverwort species from sandy soil in an openly mowed grassy area of Columbia County at Ebenezer Church. It is similar to *F. foveolata* Lindb., but is distinct in a suite of vegetative and reproductive characters. This relatively small liverwort occurs on the loose sandy to sandy loam soils that typically drain water fairly quickly and have little moisture holding capacity. This liverwort is the first dioecious species of this genus to be documented from North America. At present, *F. marshii* appears to be restricted to the Western Gulf Coastal Plain physiographic region of the state. However, future fieldwork in LA, MS and TX with a review of herbarium specimens, particularly those labeled *F. foveolata*, may result in expanding the known distribution; thus, a possible future removal from the list of Arkansas endemics.

Platyhelminthes: Monogeneoidea: Dactylogyridae

Dactylogyryus asper Chien, 1974 (a fish gill parasite).

Chien (1974) described this monogenean from Redspot Chub, *Nocomis asper* from Spavinaw Creek in Benton County, Arkansas River drainage. It may eventually be found in adjacent OK in the same drainage where *N. asper* also occurs.

Arkansas Endemic Biota

Table 1. Biota added to the state list of endemic species of Arkansas and counties of occurrence.

Taxon	County/counties	Reference
Fungi		
<i>Hymenopellis sinapicolor</i>	Saline	Peterson and Hughes (2010)
Plantae		
<i>Fossombronia marshii</i>	Columbia	Stotler et al. (2010)
Animalia		
<i>Dactylogyrus asper</i>	Benton	Chien (1974)
<i>Dactylogyrus boopsi</i>	Franklin, Fulton, Newton, Polk, Washington	Cloutman (1994)
<i>Dactylogyrus greenei</i>	Franklin, Johnson, Perry	Cloutman (1995)
<i>Dactylogyrus latrictus</i>	Izard	Chien (1974)
<i>Dactylogyrus robisoni</i>	Calhoun	Cloutman (2011)
<i>Gyrodactylus brentinae</i>	Clay	Wellborn (1967)
<i>Chaetaspis attenuatus</i>	Independence, Izard	Lewis and Slay (2013)
<i>Speleochus blanchardensis</i>	Stone	Carlton (2012)
<i>Speleochus macosar</i>	Madison	Carlton (2012)
<i>Etheostoma clinton</i>	Clark, Montgomery, Polk	Layman and Mayden (2012)
<i>Eurycea subfluvicola</i>	Hot Spring	Steffen et al. (2014)
<i>Percina brucehompsoni</i>	Clark, Montgomery, Pike, Polk	Robison et al. (2014)

Dactylogyrus boopsi Cloutman, 1994 (a fish gill parasite).

This monogenean was described by Cloutman (1994) from Bigeye Shiner, *Notropis boops*. To date, it is restricted to Franklin, Fulton, Newton, Polk and Washington counties in the Ouachita and Ozark plateaus.

Dactylogyrus greenei Cloutman, 1995 (a fish gill parasite).

Cloutman (1995) described this monogenean from Wedgespot Shiner, *Notropis greenei*. It is known only from 3 counties, Franklin, Johnson and Perry.

Dactylogyrus latrictus Chien, 1974 (a fish gill parasite).

This monogenean was described by Chien (1974) from Hornyhead Chub, *Nocomis biguttatus*. It is known only from the White River at Sylamore, Izard County.

Dactylogyrus robisoni Cloutman, 2011 (a fish gill parasite).

This monogenean was described from the gills of Bluehead Shiner (*Pteronotropsis hubbsi*) collected from Locust Bayou at AR St. Hwy. 4, 1.0 km W of Locust Bayou, Calhoun County (Cloutman 2011).

Gyrodactylus brentinae Wellborn, 1967 (a fish gill parasite).

This ectoparasite was described from the gills of Speckled Darter, *Etheostoma stigmaeum* from the W.H. Donham State Fish Hatchery at Corning, Clay County (Wellborn 1967). However, it may eventually be found elsewhere in the range of *E. stigmaeum* (Layman and Mayden 2012) in other states (AL, FL, GA, KY, LA, MO, MS, TN) since it was collected from a fish hatchery.

Arthropoda: Diplopoda: Polydesmida: Macrosternodesmidae

Chaetaspis attenuatus Lewis & Slay, 2013 (a millipede).

This cavernicolous millipede was described by Lewis and Slay (2013) from Cushman Cave in Independence County. Other specimens were taken from Clay Cave, Izard County, about 30 km NW of the type locale. These two caves occur along the northeastern side of the White River and are separated by about 100 km from a cave locality of its sister species, *C. aleyorum* Lewis in Taney County, Missouri.

Arthropoda: Hexapoda: Coleoptera: Staphylinidae

Speleochus blanchardensis Carlton, 2012 (cave pselaphine).

A staphylinid cave beetle described from “The Maze” section of Blanchard Springs Caverns, Stone County (Carlton 2012).

Speleochus macosar Carlton, 2012 (cave pselaphine).

Another cave beetle described by Carlton (2012) from Whippoorwill Cave, Madison County. Specimens were taken within 98-250 m of the entrance of the cave that measures approximately 2 km in length.

Osteichthyes: Perciformes: Percidae

Etheostoma clinton Mayden & Layman, 2012 in Layman & Mayden, 2012 (Beaded Darter).

This darter, a member of the subgenus *Doration*, was described from specimens collected in the upper Ouachita and Caddo rivers (Layman and Mayden 2012). The type locality is the Caddo River at AR St. Hwy. 182, 3.2 km N of Amity, Clark County. It also occurs in Ouachita streams in Montgomery and Polk counties. Interestingly, populations of *E. clinton* from the upper Caddo and upper Ouachita rivers are isolated from each other by several man-made impoundments.

Percina brucehompsoni Robison, Cashner & Near, 2014 (Ouachita Darter).

Robison et al. (2014) described the Ouachita Darter, which is endemic to the upper Ouachita River system. The type locality is the Ouachita River at AR St. Hwy. 298, approximately 1.6 km S of Sims, Montgomery County. This species is a sister species to the Longnose Darter (*Percina nasuta*) and also occurs in streams in Clark, Pike and Polk counties.

Amphibia: Caudata: Plethodontidae

Eurycea subfluvicola Steffen, Irwin, Blair & Bonett, 2014 (Ouachita streambed salamander).

A new species of paedomorphic salamander was recently described from a tributary of Slunger Creek, a first order stream in Lake Catherine State Park, Hot Spring County by Steffen et al. (2014). This salamander has the most restricted range of any North American amphibian and has been afforded protection from collection by various AR state agencies.

Discussion

Each of the endemic species reported herein come from at least one of 20 of 75 (27%) different counties of the state (Fig. 1). However, multiple records of endemics are from Clark (2 spp.), Franklin (2 spp.), Izard (2 spp.) and Polk (3 spp.) counties. Interestingly, the vast majority (16 total or 80%) of these counties are geographically situated in the Interior Highlands (Ozarks and Ouachitas) of Arkansas, an area known previously for high biodiversity and endemism. This is the result of climatic and geologic history of the region, having been continually habitable for all biota for about 320 million yr (Allen 1990).

In conclusion, we have added 13 endemic species to the Arkansas list bringing the total number of endemics in the state to 139. The number of endemic species has increased dramatically since 1974, when the first list was compiled (Table 2). In addition, we are aware of a new trichopteran from the Saline River, Saline County (Etnier 2010), a new anilline ground beetle from Blanchard Springs, Stone County (C. Carlton, *pers. comm.*), and various cavernicolous species, including a new cavefish (Graening et al. 2011), that await formal description. Given the taxonomic breadth gained on flora and fauna from molecular techniques, as well as continued exploration

Table 2. Comparative data on number of endemic biota (taxa) of Arkansas 1974-present.*

Total Endemic Taxa	Fungi	Plantae	Animalia	Reference
9	0	4	5	Arkansas Department of Planning (1974)
47	0	7	40	Robison and Smith (1982)
85	0	9	76	Allen (1988)
117	0	11	106	Robison and Allen (1995)
113	0	10	103	Robison et al. (2008)
126	2	10	114	McAllister et al. (2009)
139	3	11	125	Robison and McAllister (this report)

*List does not include bacteria, Cyanobacteria, Archaea, and Protista; we are aware of several endemic taxa in these groups, particularly coccidian parasites (Protista: Apicomplexa).

Arkansas Endemic Biota

of caves that yield obligate subterranean biodiversity (Graening et al. 2011), additional species will undoubtedly be added to future lists.

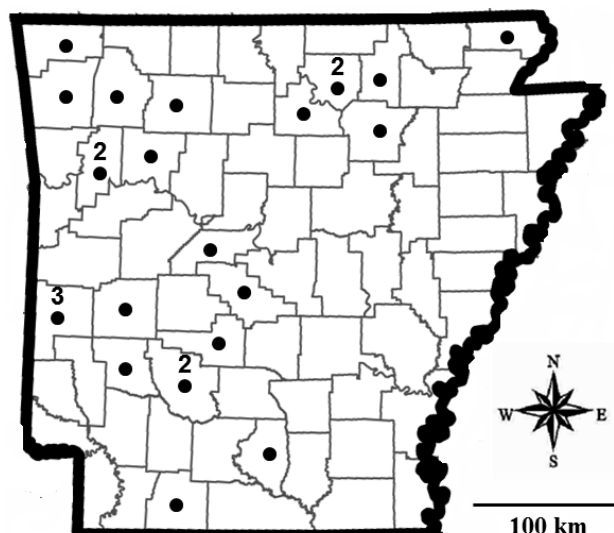


Figure 1. Counties with records of the endemic species reported herein. Numbers above dots represent more than one endemic species record from the particular county.

Acknowledgments

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Red Crossbill Invasion of Northwestern Arkansas During 2012-2013

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Running head: Red Crossbill Invasion During 2012-2013

Abstract

An irruption of Red Crossbills (*Loxia curvirostra*) occurred in primarily northwestern Arkansas starting in November of 2012 and lasting to the end of May of 2013. Based on recordings of call notes, most birds around Fayetteville were Type 2, the large-billed ponderosa pine crossbill, associated with a variety of conifer species. Birds recorded in Carroll County were Type 3, the small-billed western hemlock crossbill, and they were associated with small cones on shortleaf pine (*Pinus echinata*). One recording was obtained in Fayetteville of Type 5, the lodgepole pine crossbill, only the third recording east of the Great Plains. Crossbills at the Fayetteville Country Club were observed eating algae (*Cladophora* sp.) during the months of December and January, a behavior rarely reported for passerines. During March, crossbills appeared at sunflower bird feeders, which is a relatively recent phenomenon associated with low conifer seed abundance. The first two Arkansas specimens of crossbills (probably Type 3) were obtained from birds that struck windows near feeders. This is only the third recorded irruption of crossbills in Arkansas in the last 43 years, suggesting that crossbills rarely travel this far south in search of cone crops.

Introduction

Red crossbills (*Loxia curvirostra*) have been reported in every month of the year and have possibly bred in Arkansas, but are an irregular visitor to state (Arkansas Audubon Society 2014). The first mention is of numerous birds near Clinton (Van Buren County) in spring of 1889 and one bird in spring of 1890 (Howell 1911). Over the next 60 years, only 2 more sightings were reported: Monte Ne (Benton County) in February and March of 1932 and Monticello (Drew County) in spring of 1934 (Baerg 1951). Starting in 1969, birds have been seen sporadically near Lake Georgia Pacific

(Ashley County), primarily on Christmas Bird Counts (James and Neal 1986, Fig. 1).

Crossbills are considered to be an irruptive species, wandering great distances in search of cone crops when cone crop failures occur within their normal range (Adkisson 1996). Currently 10 call types are recognized which may relate to geographical and reproductive isolation and specialization on one or a suite of conifer species (e.g., Benkman 2007, Young 2011). Although specialized on one species, crossbill types can feed on other conifers opportunistically.

The first documented irruption in Arkansas was from September 1972 to April 1973 (James and Neal 1986). Another irruption occurred from January 1997 to June 1997 (Arkansas Audubon Society 2014). We document a third irruption that began in November 2012 and lasted to the end of May 2013 primarily in northwestern Arkansas. We recorded birds to identify call types, made observations on behaviors of the crossbills, and obtained the first specimens for the state.

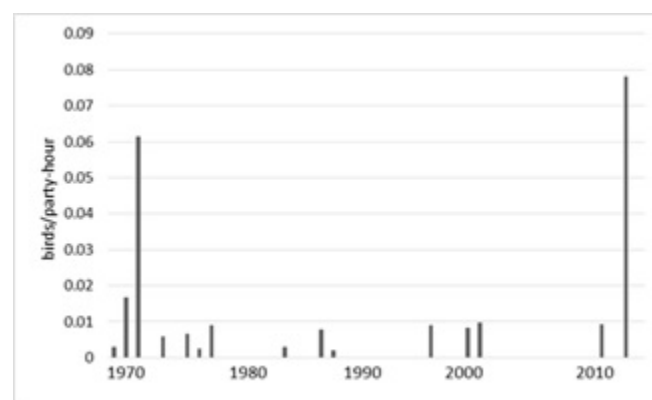


Figure 1. Number of Red Crossbills seen on Christmas Bird Counts in Arkansas from 1969, the first year they were recorded, through 2013, standardized by birds/party-hour. The greatest irruption was in 2012-2013, followed by the one in 1972-1973. The irruption of 1997 started in January after the bird counts had been completed. (data: <http://netapp.audubon.org/cbcobservation/>)

Methods

The chronology of the irruption was monitored primarily through ARBIRD-L, a list server about Arkansas birds maintained by Smith. This included both information on movement of birds and their behavior.

Birds were recorded by Neal using a Sony linear PCM recorder PCM-M10. He recorded birds at the Fayetteville Country Club (FCC) (Washington County) on 7 and 10 December 2012 and 20 January 2013, at the Ninestone Land Trust (Carroll County) on 26 and 27 January and 9 February, at the University Farm in Fayetteville (Washington County) on 5 February, and at the Ozark Natural Science Center and adjacent McIlroy Madison County Wildlife Management Area (Madison County) on 19 February. Don Matt and Judith Griffith, owners of Ninestone Land Trust, also made some recordings. All recordings were analyzed and call types identified by Young. Birds were confirmed to call types via audiospectro-graphic analysis using Raven Pro 1.5 (Cornell Laboratory of Ornithology, Ithaca, NY).

Two specimens were obtained when birds were killed hitting windows near feeders. They were frozen and donated to the collection at the Louisiana Museum of Natural History at Louisiana State University. Donna Dittmann prepared the specimens and Steven Cardiff measured bill depth.

Results

Chronology of the Irruption

On 12 November 2012, a flock of 30 Red Crossbills was observed by Neal at Brentwood (Washington County). The following day, he observed a flock of 10 birds at the FCC. On 30 November, Neal found a flock of 6 birds at Shores Lake (Franklin County) in the Ozark National Forest. Scattered reports were made in other parts of the state, e.g. 4 birds at Toad Suck Ferry (Faulkner County) by Michael Linz and 6 by Leif Anderson at Felsenthal National Wildlife Refuge in south central Arkansas, both on 19 December. However, the vast majority of reports were confined to Benton, Washington, Madison, and Carroll counties in northwestern Arkansas.

High counts of crossbills were of over 50 birds at the FCC on 20 January and 10 March 2013. Flocks of 15-25 birds were seen at Ninestone Land Trust in January and February and a flock of 28 birds was seen on 17-18 February at the Ozark Natural Science

Center. The highest number of crossbills (74) ever recorded on Arkansas Christmas Bird Counts occurred during this irruption (Fig. 1). The last report was of a single female at a bird feeder at Hobbs State Park – Conservation Area (Benton County) on 27 May 2013.

Call Types

The vast majority of recordings were of Type 2 – the ponderosa pine crossbill. All calls recorded from a flock of 23 birds on 7 December at the FCC were Type 2, 17 of which were the kinked call associated with crossbills from western United States (Young 2012). Most calls recorded from a flock of 30 birds at the FCC on 10 December and all from a flock of 50 birds there on 20 January were also Type 2. All birds recorded from a flock of 14 at the University Farm in Fayetteville on 2 February were Type 2, as were those from a flock of 28 birds recorded at the Ozark Natural Science Center on 18 February.

On 10 December, a few Type 3 crossbills – the western hemlock crossbill – were recorded at the FCC in a flock with Type 2 calls (Fig. 2). Type 3 was the only crossbill recorded at Ninestone Land Trust on 26 and 27 January and on 9 February.

A third type crossbill was recorded at the FCC on 10 December (Fig. 3). Type 5 – the lodgepole pine crossbill – has rarely been reported east of the Great Plains.

Feeding on Algae

On the morning of 9 December, Smith observed small flocks of both male and female crossbills foraging on mats of algae on the banks of a pond at the FCC, apparently eating algae (Fig. 4). Due to a drought, the pond was lower than normal and had large areas of dried and drying green algae (*Cladophora* sp.). Birds were observed foraging on the mats and eating

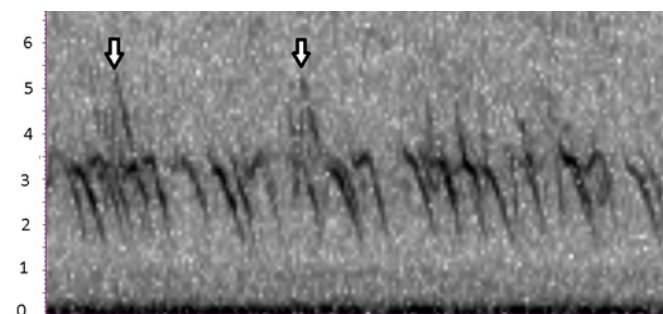


Figure 2. Spectrogram of Red Crossbill calls recorded at the Fayetteville Country Club on 10 December 2012. Most of the calls are of Type 2, but 2 of the higher frequency Type 3 calls can be seen (arrows). Y-axis is in kilohertz.

Red Crossbill Invasion During 2012-2013

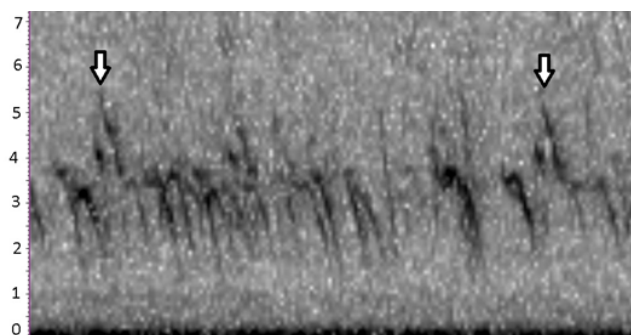


Figure 3. Spectrogram of Red Crossbill calls recorded at the Fayetteville Country Club on 10 December 2012. Most of the calls are of Type 2, but the higher pitched Type 5 call can be seen (arrows). Y-axis is in kilohertz.

algae through January 2013, usually in the mornings by numerous observers.

Moving to Sunflower Feeders

In mid-March, people started reporting Red Crossbills at bird feeders containing sunflower seeds throughout Benton and Washington counties. Birds were commonly seen at feeders during April and early May.

Specimens

The first two Arkansas specimens of Red Crossbill were obtained from birds that struck windows near feeders: a female was collected by Kathy Ross on Redbud Lane in Rogers (Benton County) on 9 April 2013 and a male was collected by Betty and Wes Whittington on Gary Turner Road in Siloam Springs (Benton County) on 15 April. At the time of preparation, the female (LSUMZ 184762) weighed 25.9 g and the male (LSUMZ 184763) weighed 29.9 g. The bill depth of both birds was 8.6 mm. Putatively, these 2 specimens are Type 3 as their bills are too small to be Type 2 (see Benkman et al. 2009), but are within the range of Type 3 bill sizes (e.g., Groth 1993).

Discussion

Our spectrographic analyses suggest the most common call type during the invasion was Type 2. The large-billed Type 2 form is generally widespread, e.g., it was found in several locations in both the Great Plains and the Northeast during fall of 2012 and spring of 2013 (M. A. Young, *unpubl. data*). The ubiquity of Type 2 may be due in part to their ability to utilize a variety of conifer species (Groth 1993) as they are capable of eating both soft and hard cones. Type 2 birds were found in a variety of conifer species at the

FCC; they used shortleaf pines (*Pinus echinata*) at the Ozark National Science Center.



Figure 4. Red Crossbills eating algae (*Cladophora* sp.) at the Fayetteville Country Club on 10 December 2012. This behavior continued for nearly 2 months.

Type 3 was also wide spread during the same time period (M. A. Young, *unpubl. data*), waging a massive large-scale irruption from coast to coast starting summer of 2012 and lasting through spring 2013 (e.g., Kolbe and Brinkley 2013). Although detected early in the irruption at the FCC, this type persisted at the Ninestone Land Trust in stands of shortleaf pine. Type 3 has the smallest bill of any North American crossbill type and shortleaf pine has the smallest cone of any typical conifer found in the southeastern United States.

The detection of call Type 5 is only the third record of this type east of the Great Plains. The first was in New York in 2006 (Young 2010). The second record occurred one week earlier than ours on 2 December 2012 at Rocky Fork Lakes Conservation Area (Boone County) in Missouri (<http://ebird.org/ebird/view/checklist?subID=S12204977>). Lodgepole pine (*Pinus contorta*) forests in western United States are being decimated by the mountain pine beetle (*Dendroctonus ponderosae*) (Man 2012), which may account for the eastern movement of this call type. The brief appearance of Types 3 and 5 at the FCC may be related to the difficulty these smaller crossbills would have finding suitable cones to forage on (C. Benkman, *pers. comm.*).

Passerines have rarely been reported to eat algae and this is the first report of crossbills eating algae. American Goldfinches (*Spinus tristis*) are the only other passerines reported to consume algae (*Spirogyra*

sp.) in North America (Digioia 1974, Kilham 1988:124-125). Why crossbills were eating algae remains unclear. *Cladophora* harbors a wide variety of insects (A. Alverson, *personal communication*), and crossbills do eat insects (Groth 1993:89), but usually in spring and summer (Adkisson 1996). We speculate that, due to a possible cone shortage, birds supplemented their diet with algae. Dried algae may provide crossbills with salt, a known attractant, and grit, a dietary requirement (Adkisson 1996). On 11 February 2013, 2 crossbills were observed on the ground eating red soil at the FCC by Neal.

The use of bird feeders by Red Crossbills is a relatively recent phenomenon (Benkman 2011). Periods of low seed availability (e.g., late spring and summer) could leave crossbills food stressed. Benkman (1988) demonstrated that crossbills can consume sunflower seeds, but their intake rate has to be quite high to substitute for conifer seeds. Benkman (2011) speculated that current increases in temperature may cause conifers to drop their seeds earlier, further stressing crossbills. Bird feeders with sunflower seeds may be one of the only food sources available to crossbills before insects become available. Crossbills will also eat tree buds and were observed doing that in mid-April at the FCC (M. Pruitt, *pers. observ.*).

The first documented irruption in Arkansas occurred in 1972-1973, starting in September and lasting to April (James and Neal 1986). This coincided with a larger-spread irruption of several species across the United States (Koenig and Knops 2001) and could have been associated with the massive eastern spruce budworm (*Choristoneura fumiferana*) outbreak in eastern Canada at that time (Bolgiano 2004). The second irruption in 1997 was unusual in that it did not start until January, but lasted until June (Arkansas Audubon Society 2014). This also was a geographically wide-spread irruption with many crossbills throughout the Northeast (Bolgiano 2004, Young 2011). This third irruption started in November of 2012 and lasted until May of 2013, and was wide-spread across the eastern United States. Irruptions of western crossbill call types into the eastern United States suggest cone crop failures in the West as the cause. Furthermore, the paucity of crossbill irruptions in Arkansas in the past 43 years suggest that crossbills usually find large cone crops to the north when they wander east, and rarely search as far south as Arkansas, at the southern edge of their range (see map in Adkisson 1996).

Acknowledgments

We thank the many people that contributed information on the irruption either directly to us or through ARBIRD-L. Andy Alverson identified the algae. Craig Benkman offered advice on several aspects of the research. He and an anonymous reviewer offered helpful suggestions on the manuscript. This paper is dedicated to the memory of Curtis Adkisson, a native Arkansan who contributed much to the study of crossbills.

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Selection of a Realistic Viscous Vortex Tangential Velocity Profile for Computer Simulation of Vortex-Structure Interaction

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Running Title: Selection of a Realistic Viscous Vortex Tangential Velocity Profile

Abstract

Structure loading by vortices is a relevant phenomenon in numerous fields of engineering significance. Computer modelling is a powerful tool that can be used to study the loading produced on structures by impacting vortices. Realistic simulation of vortex-loading of structures necessitates the use of a realistic vortex tangential velocity profile (TVP). The present study compiles measured TVPs from various types of experimentally-produced vortices as well as real-world tornado and hurricane vortices. The measured TVPs are compared with commonly-used, analytical TVPs. Analytical TVPs that realistically represent the range of measured TVPs are identified and selected for use in future computer simulation studies.

Introduction

Viscous vortices are complex flow phenomenon that are studied for numerous engineering applications. The aerospace community seeks to mitigate interaction between shed vortices and rotors of helicopters which produces impulsive noise and vibration (Ramasamy and Leishman 2006). Also, wings of flapping micro-air vehicles are designed for thrust enhancement due to interaction between wing tip and leading edge vortices (Ellington 1999). The civil and structural design communities seek to develop accurate design loadings for tornado wind loads on buildings (Selvam and Millet (2003, 2005), Sengupta et al. (2008), Haan et al. (2010)). Finally, meteorologists seek to predict the growth and trajectory of hurricanes and tornados so that advance warning can be given to surrounding areas (Cao et al. 2011).

Most vortices of engineering interest are “intense” meaning the tangential velocity V_θ is much greater than the radial or axial velocity (Vatistas 1998). It is generally accepted that the cross section of the vortex consists of three regions (Figure 1a): (1) laminar inner

core, (2) transition region, and (3) turbulent exterior. Several typical radial tangential velocity profiles (TVPs), or $V_\theta(r)$, are illustrated in Figure 1b. $V_\theta(r)$ increases moving away from the vortex center ($r < r_c$) and reaches its maxima “ $V_{\theta,max}$ ” at the critical radius $r = r_c$. Subsequently, $V_\theta(r)$ decays with increasing radial distance from the vortex center ($r > r_c$).

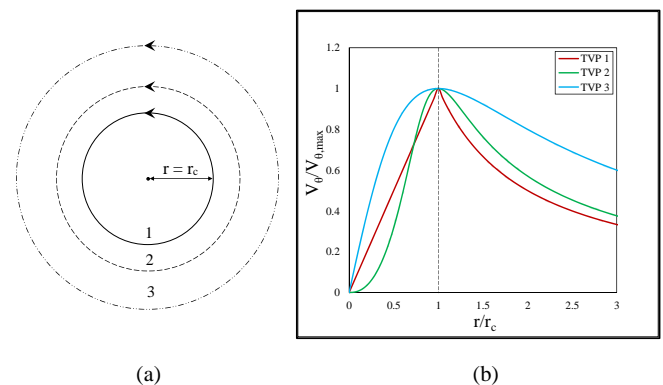


Figure 1. (a) Schematic of the 3 cross-sectional regions of the vortex and (b) illustration of 3 analytical vortex TVPs.

Extensive research has focused on defining the correct TVP for real-world, viscous vortices. Vortices are typically classified using the vortex Reynolds number $Re_v = \Gamma_\infty/\nu$, where $\Gamma_\infty = V_{\theta,max} \cdot r_c \cdot 2\pi$ is the maximum circulation in the vortex and ν is kinematic viscosity. Vatistas (2006) studied rotor tip vortices and concluded that $V_\theta(r)$ should “flatten” as Re_v increases due to turbulent diffusion of the vortex; progressive flattening of TVPs is illustrated from TVP1 to TVP3 in Figure 1b. However, Kessler (1970) notes that the TVP may not just be a function of Re_v , as similar-sized tornados may have laminar or turbulent structure as illustrated in Figures 2a and 2b respectively. The Doppler on Wheels (DOW) group has recorded tornado TVPs since 1995, hence they are the primary source for field-measured tornado data. Even with advancements in radar capabilities, their lowest

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reported measurements of tornado TVPs are ≈ 40 m above ground level (Kosiba and Wurman 2010). As previously concluded in Wurman et al. (2007), the current understanding of near-ground tornado TVPs is at best an educated guess. Kepert (2010) reaches a similar conclusion for hurricanes, noting that $V_\theta(r)$ may vary from v- to u-shaped (TVP1 to TVP2 in Figure 1b) depending upon numerous environmental parameters.



(a) (b)

Figure 2. (a) Laminar (TornadoFacts, 2009) and (b) turbulent (Zimmerman, 2012) tornado vortex.

Meaningful computer simulation of vortex-structure interaction, at both the rotor tip vortex and tornado vortex scales, necessitates the use of analytical TVPs that give realistic representation of real-world viscous vortices. The viscous vortex is an extremely complex phenomenon, the physics of which are clearly not well understood. However, the literature documents measured TVPs from both laboratory-generated and convection-driven vortices. The best approach to select analytical TVPs for computer simulations is to assimilate measured TVPs and identify the analytical TVPs that provide the best representation of the measured data.

The present work collects and compares viscous vortex TVPs reported in the literature. Measured TVPs are grouped into 6 categories by vortex and experiment type. Analytical TVPs are then introduced and compared with the measured TVPs. Analytical TVPs which best fit the measured TVPs are identified and recommended for use in computer simulation of vortex-structure interaction.

Measured Tangential Velocity Profiles

Vortex Chamber Experiments

Vortex chamber experiments are commonly used to investigate flows in vortex combustors and

separators (Vatistas et al. 1986). Generally stated, fluid is input at one end of the chamber as four tangential streams spaced at $\pi/2$ around the circumference of the chamber and extracted as a single axial stream at the opposite end of the chamber.

Pritchard (1970) employs a different experimental method than that used by the other four sources. A cylindrical bucket is filled with water seeded with reflective spheres. The water is then stirred, and streak photography is used to compute the TVP.

Parameters for the vortex chamber experiments are not well reported. Faler and Leibovich (1977) report a Reynolds number range of $3000 \leq Re \leq 6000$, but do not explain how they defined the Reynolds number. It is believed, however, that the vortex chamber vortices have lower Re_v compared with the tornado simulator profiles discussed subsequently. Summary of the measured TVPs is provided in Figure 3.

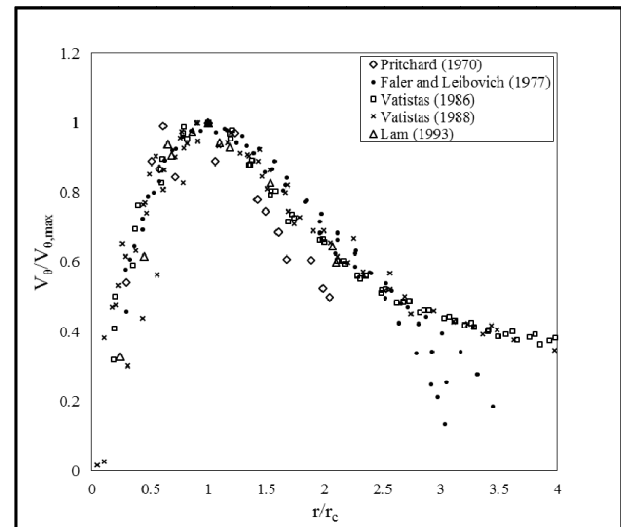


Figure 3. Measured TVPs from vortex chamber experiments.

The vortex chamber TVPs are all relatively well grouped. Pritchard's (1970) TVP falls below the TVPs; this is likely due to the inferior data collection method (streak photographs instead of pressure probes) that is used. Faler and Leibovich's (1977) TVP exhibits unrealistic, rapid decay for $r/r_c > 2.5$. It is postulated that they report measurements taken too closely to the walls of the chamber, hence the vortex is damped by the confining walls.

Tornado Simulator Experiments

Tornado simulators are used to study both the structure of tornados (Church and Snow 1993) and the

structure loading they produce (Haan et al. 2010). Generally stated, a large blower or fan is mounted at the top of a hood, and fluid is pulled into the hood through many angled vanes spaced around the circumference of the hood. The hood may be stationary (Wilkins (1964), Wan and Chang (1972)) or may translate (Kuai et al. (2008), Haan et al. (2010)).

The translating tornado simulator at Iowa State University is the current standard for tornado simulators. Haan et al. (2008) provide further details of the design and testing of the Iowa State tornado simulator. Measured TVPs from tornado simulators are summarized in Figure 4.

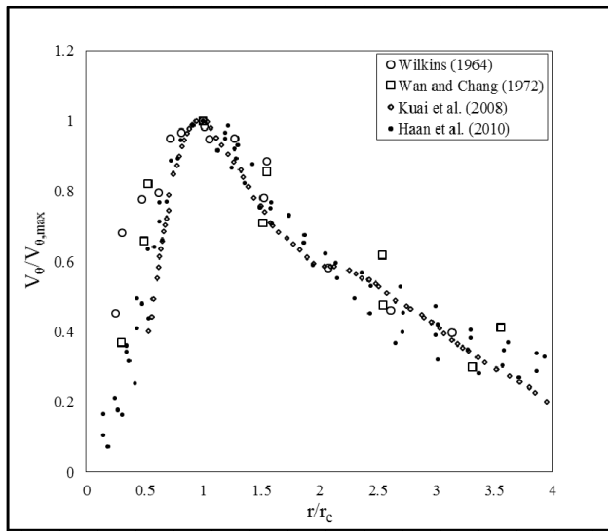


Figure 4. Measured TVPs from tornado simulator experiments.

Tornado simulator experiments are compared using the previously-defined Re_v . Some in the literature prefer to discuss experimental vortices in terms of the swirl ratio S , which is the ratio of the tangential velocity to the axial (or vertical) velocity of the vortex. Given the swirl ratio and volumetric flow rate Q through the fan, the total circulation is defined as $\Gamma_\infty = 2 \cdot S \cdot Q \cdot r_c^{-1}$. Summary of Re_v for the tornado simulator experiments is provided in Table 1.

Table 1. Re_v range for tornado simulator experiments

Source	Re_v
Wilkins (1964)	205,000
Wan and Chang (1972)	710,000 to 1,300,000
Kuai et al. (2008)	1,798,000 to 2,062,000
Haan et al. (2010)	1,800,000 to 4,165,000

The tornado simulator experiments represent a wide range of Re_v . There is some scatter in the data, but all sets exhibit the same trend. An interesting observation is that the TVP decays more sharply in the tornado simulator experiments than the TVP decays in the vortex chamber experiments. This seems to disagree with the view that the TVP should flatten with increasing Re_v (Vatistas 2006).

Fixed Wing Experiments

Vortices produced by fixed wings are typically studied in the aerospace community to evaluate air loads on trailing aerospace vehicles due to vortices shed from leading aerospace vehicles (Dosanjh et al. 1962). The fixed wing configuration is also used as a less complex alternative to rotor experiments. Generally stated, a wing or air foil is rigidly fixed and a stream of fluid is circulated over it. Vortices are “tripped” by movement of the wing or by some other mechanism. Measured TVPs from fixed wing experiments are summarized in Figure 5.

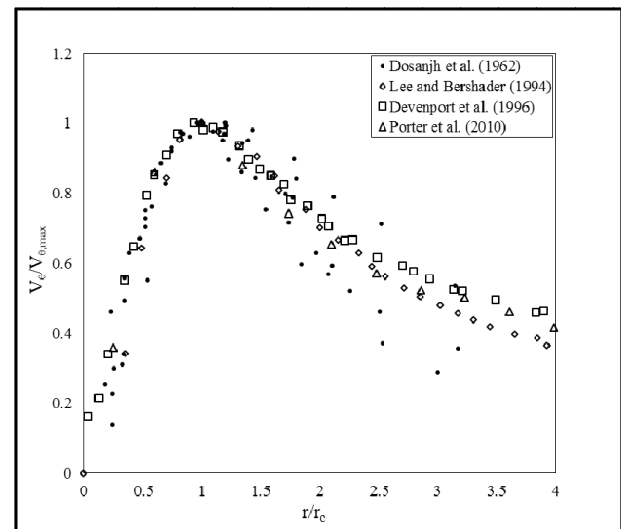


Figure 5. Measured TVPs from fixed wing experiments.

Fixed wing experiments are classified using the chord Reynolds number $Re_c = c \cdot U_\infty / \nu$, where c is the chord of the wing and U_∞ is the free stream velocity. Summary of Re_c for the fixed wing TVP experiments is provided in Table 2.

The fixed wing experiments span a relatively wide range of Re_c . All data are well grouped for $r \leq r_c$, but the TVP of Dosanjh et al. (1962) increasingly deviates from the other data sets for $r > r_c$. This could be partially due to the fact that Re_c of Dosanjh et al.

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(1962) is much lower than Re_c used in the other works. It is also noted that the fixed wing TVPs resemble the vortex chamber TVPs much more so than the tornado simulator TVPs.

Table 2. Re_c for fixed wing experiments

Source	Re_c
Dosanjh et al. (1962)	10,000
Lee and Bershader (1994)	900,000 to 1,300,000
Devenport et al. (1996)	318,000 to 742,000
Porter et al. (2010)	830,000

Rotor Tip Experiments

Vortices produced by rotors are primarily studied in the aerospace community to reduce vibration in, and noise produced by helicopters (Ramasamy and Leishman 2004). In general, a single- or dual rotor is driven by a motor, and vortices are tracked in the rotor wake. The single-rotor configuration is the most commonly-observed configuration. Summary of measured rotor tip TVPs is provided in Figure 6.

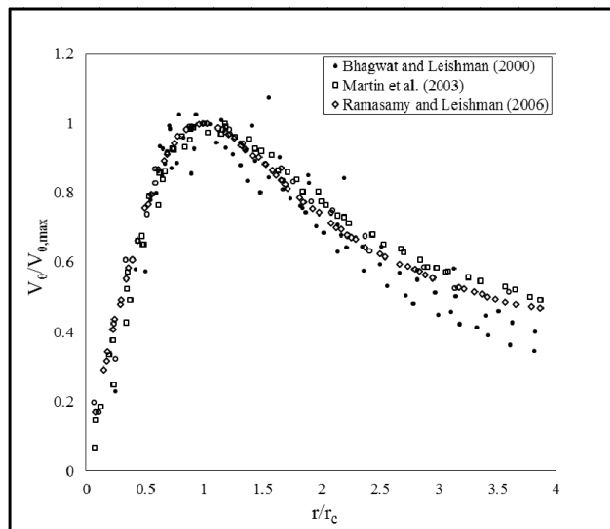


Figure 6. Measured TVPs from rotor tip experiments.

Rotor tip vortices are characterized using Re_c , which is previously defined for fixed wing experiments. Summary of Re_c for the rotor tip experiments is provided in Table 3. Note that all of the reported rotor tip experiments are performed by JG Leishman's group at the University of Maryland using the same experiment configuration.

Table 3. Re_c for rotor tip experiments

Source	Re_c
Bhagwat and Leishman (2000)	270,000
Martin et al. (2003)	272,000
Ramasamy and Leishman (2006)	272,000

Within measurement error, it can be assumed that all of the rotor tip experiments are conducted for the same Re_c . Ramasamy and Leishman (2004) define the vortex Reynolds number for their experiment to be $Re_v = 48,000$. This implies that the vortices produced by the tornado simulator ($205,000 \leq Re_v \leq 4,165,000$) are much more turbulent, hence their TVPs should be much flatter than fixed wing and rotor tip TVPs (Vatistas, 2006). However, the measured TVPs surveyed up to this point suggest that there may not be such a direct relationship between TVP shape and Re_v .

Measured Tornado

Tornado vortices are studied to better understand the loading that they place on structures. Physical measurement of wind fields within a tornado is very hazardous, in addition to the fact that it is difficult to know when and where a tornado will occur. Early measurements of TVPs in tornados (Hoecker 1960) and water spouts (Golden 1974) use successive, timed photographs debris in funnel clouds to compute approximate wind speeds. The current standard in tornado TVP measurement is high resolution, mobile W-band Doppler radar ($\lambda = 3 \text{ mm}$, $f = 95 \text{ Hz}$), which is used in the other four works. A summary of measured tornado TVPs is provided in Figure 7.

As might be expected, the field-measured tornado TVPs exhibit much greater variation than any of the previously-considered experimental data sets. The early TVP measurements (Hoecker 1960, Golden 1974) computed from photographs of debris employ excessive estimation, hence it seems reasonable that these measurements should differ from the later and more accurate radar measurements. However, there is even considerable scatter within the radar-measured TVPs. A summary of the details of the radar-measured TVPs is provided in Table 4.

Radar measurements lose accuracy due to two primary factors: attenuation of the emitted signal and increased observation distance. Signal attenuation occurs due to absorption and scattering, both of which are enhanced by moisture and contaminants in the air. As the observation distance increases, the area covered

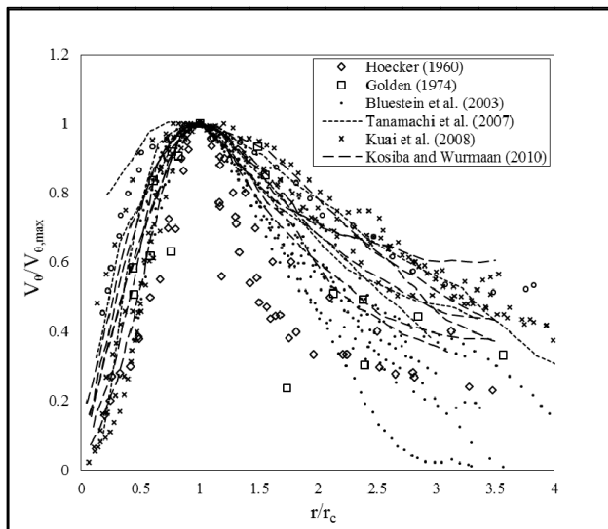


Figure 7. Measured TVPs for tornado field data.

Table 4. Measurement details for field-measured tornados

Source	Measurement	
	Height (m)	Distance (km)
Bluestein et al. (2003)	N/A	2.3 - 7.0
Tanamachi et al. (2007)	70 - 155	4.5 - 6.8
Kuai et al. (2008)	20 - 660	1.7 - 12.9
Kosiba and Wurman (2010)	≈ 40	1.7 - 6.5

by the emitted pulse increases, hence resolution of the radar image decreases. Furthermore, the signal must travel over a greater distance leading to greater attenuation of the signal. The high-frequency W-band is used because it is able to provide high temporal resolution of the tornado structure. However, as the wavelength of a signal shortens, it is attenuated much more rapidly. In short, although the radar-measured TVPs are measured at similar distance and with the same radar technology, many factors can influence and distort the measured TVP. Different levels of moisture and or dust in the air surrounding the vortex may substantially influence the measured TVP and is likely the cause of the substantial deviation in the measured tornado TVPs.

Measured Hurricane

Hurricane TVPs are primarily studied to allow forecasting of their size and trajectory (Cao et al. 2011). Because hurricanes are large and slow-moving,

they can be tracked for days or even weeks before making landfall. Early measurements of hurricane TVPs were made by manned flights through the eye-wall of the hurricane as summarized in Willoughby (1990). Manned flight through a hurricane is hazardous to human life, hence alternative measurement procedures have been developed. The current standard in measurement of hurricane properties is via dropsonde. Specifically, manned or unmanned aircraft fly above the hurricane and seed it with numerous data-acquisition dropsondes. These are equipped with GPS and report local velocity and pressure at specified heights. Summary of measured hurricane TVPs is provided in Figure 8.

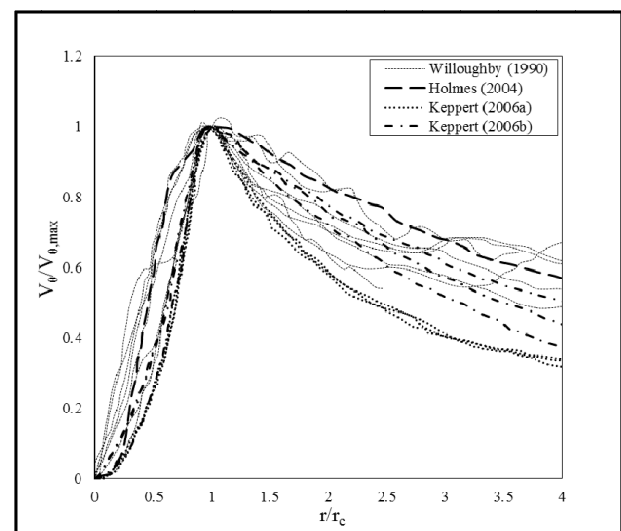


Figure 8. Measured TVPs for hurricanes.

Generally, the hurricane TVPs are well grouped with no significant outlying data. Keppert reports TVPs measured at 500, 1000, and 2000 m for two separate hurricanes. His first study shows that the TVP remains relatively constant with increased elevation (Keppert 2006a). His subsequent study, however, shows that the TVP decays more slowly with increasing elevation (Keppert 2006b).

Analytical Tangential Velocity Profiles

Numerous analytical TVPs for viscous vortices are discussed in the literature. Bhagwat and Leishman (2002) survey TVPs for rotor tip and fixed wing applications and more recently in Wood and White (2011) survey TVPs for tornados and hurricanes. The present study is only concerned with comparing the analytical TVPs with the measured TVPs, hence the

Selection of a Realistic Viscous Vortex Tangential Velocity Profile

assumptions and derivations of the analytical models shall not be discussed. However, the interested reader can find these details in the cited works.

First, analytical TVPs derived from the Navier Stokes equations are introduced. It is important to include the original names of these profiles, as these names are primarily used in the literature. Subsequently, two algebraic TVPs which are used to reproduce the derived TVPs are introduced and discussed. The capability of the algebraic profiles to reproduce the derived TVPs is then demonstrated.

Derived Tangential Velocity Profiles

The most commonly-used analytical TVPs for fixed-wing and rotor-tip vortices are the Lamb-Oseen (L-O) (Ramasamy and Leishman 2006) and Scully-Kaufmann (S-K) (Vatistas 2006) profiles defined by equation (1) and equations (2-3) respectively.

$$V_{\theta,S-K}(r) = r \cdot r_c \cdot (r^2 + r_c^2)^{-1} \quad (1)$$

$$V_{\theta,L-O}(r) = r_c / r \cdot [1 - \exp(-\alpha \cdot r^2 / r_c^2)] \quad (2)$$

$$r_c(t) = (4 \cdot \alpha \cdot v \cdot t)^{0.5} \quad (3)$$

The Oseen constant in equations (2-3) is $\alpha = 1.25643$. Also note that the L-O vortex stretches in time due to the viscosity of the fluid. The present work is concerned only with the profile shape, hence r_c is fixed. When r_c is fixed in equation (2), the L-O profile is identical to the Burgers-Rott profile.

TVPs of atmospheric vortices are most commonly modeled using the modified Rankine (MRCVM), Burgers-Rott (B-R), or Sullivan (S) profiles (Wood and White 2011). The MRCVM is a bi-regional profile defined using equations (4-5). The value of the exponent in equation (5) ($r > r_c$) varies in the literature from $0.4 \leq x \leq 1.0$ (Kosiba and Wurman, 2010).

$$V_{\theta,MRCVM}(r) = r / r_c \quad 0 \leq r / r_c \leq 1.0 \quad (4)$$

$$V_{\theta,MRCVM}(r) = (r_c / r)^x \quad r / r_c > 1.0 \quad (5)$$

The B-R TVP is identical to equation (2) when r_c is constant, hence re-definition is not necessary. The original Sullivan TVP is simplified by Vatistas (1998) and reported in the simplified form in equations (6-7). Note that constants $\beta = 6.238$ and $\Phi = 37.9043$.

$$V_{\theta,S}(r) = r_c / r \cdot [H(\beta \cdot (r / r_c)^2) / \Phi] \quad (6)$$

$$H(x) = \int_{0-x} \exp\{-\tau + 3 \cdot \int_{0-\tau} [(1 - \exp(-\tau)) / \tau]\} d\tau \quad (7)$$

Computation of $V_{\theta,S}(r)$ requires numerical integration of equation (7) for each radial ordinate, making the Sullivan profile cumbersome to define. Furthermore, the tornado model used by Selvam's group introduces the vortex into the domain through boundary conditions. Computation of the Sullivan profile for each boundary node via numerical integration at each time step would be very computationally expensive, hence it is not a viable option. Fortunately algebraic approximations have been developed as shall be discussed subsequently.

Algebraic Tangential Velocity Profiles

Vatistas et al. (1991) introduce the "n-family" of TVPs defined in equation (8). The exponent "n" is varied to duplicate the previously-defined analytical TVPs: S-K ($n = 1$), L-O/B-R ($n = 2$), and MRCVM ($n = 100$ for $x = 1$). This profile is robust and particularly useful in computer simulations, because a single TVP model can be incorporated and easily modified to study vortices having a range of TVP structures.

$$V_{\theta,V}(r) = r \cdot r_c \cdot ((r^{2n} + r_c^{2n})^{1/n})^{-1} \quad (8)$$

Wood and White (2011) modify the Vatistas et al. (1991) profile, adding two additional exponents to allow greater control of the TVP. One noted benefit of the Wood-White (W-W) profile is the capability to produce inner curvature in the region $r < r_c$. Consequently, the W-W profile can be used to reproduce the Sullivan TVP without requiring the numerical integration of equation (7). The W-W profile is defined in equation (9), and exponent values that reproduce the previously-defined, derived TVPs are summarized in Table 5.

$$V_{\theta,W-W}(r) = (r \cdot r_c)^{\kappa} \cdot [1 + \kappa / \eta \cdot ((r / r_c)^{\kappa / \psi} - 1)]^{-1} \quad (9)$$

Table 5. W-W exponents to approximate derived TVPs

Profile	κ	η	ψ
S-K	0.850	1.700	0.700
L-O/B-R	1.000	2.265	0.830
Sullivan	2.401	3.433	0.435
MRCVM ($x = 1$)	1.000	2.000	0.010

Profile Normalization and Comparison

All of the analytical vortex profiles defined previously reach maximum tangential velocity at the critical radius, restated $V_{\theta}(r_c) = V_{\theta,max}$. However, not all

of the analytical profiles reach the same value of $V_{\theta, \max}$. For meaningful comparison of the analytical profiles, as well as comparison of the analytical and measured profiles, it is necessary to normalize the analytical profiles such that $V_{\theta}(r_c) = 1$.

The MRCVM profile (equations (4-5)) and the W-W profile (equation (9)) are already normalized. However, the S-K, L-O, and Vatistas profiles need to be normalized. Equations (1), (2), and (8) are evaluated at $r = r_c$, and the results summarized in equations (10-12).

$$V_{\theta, S-K}(r_c) = 0.5 \quad (10)$$

$$V_{\theta, L-O}(r_c) = 1 - \exp(-\alpha) \quad (11)$$

$$V_{\theta, V}(r_c) = 2^{-1/n} \quad (12)$$

Now the respective S-K, L-O, and Vatistas TVPs are normalized by dividing the original profile definitions by equations (10-12) respectively. The resulting TVPs, equations (13-15) are marked by an asterisk indicating that $V_{\theta}(r_c) = V_{\theta, \max} = 1$.

$$V_{\theta, S-K}^*(r) = 2 \cdot r \cdot r_c \cdot (r^2 + r_c^2)^{-1} \quad (13)$$

$$V_{\theta, L-O}^*(r) = (1 - \exp(-\alpha))^{-1} \cdot r_c / r \cdot [1 - \exp(-\alpha \cdot r^2 / r_c^2)] \quad (14)$$

$$V_{\theta, V}^*(r) = r \cdot r_c \cdot (2 / (r^{2n} + r_c^{2n}))^{1/n} \quad (15)$$

The normalized TVPs are compared with approximations by the algebraic profiles in Figures 9a and 9b. The W-W approximation of Sullivan's profile is plotted as well. The exact solution to equation (6) is not provided for comparison, but Wood and White (2011) show that their approximation has RMS error of only 0.0005.

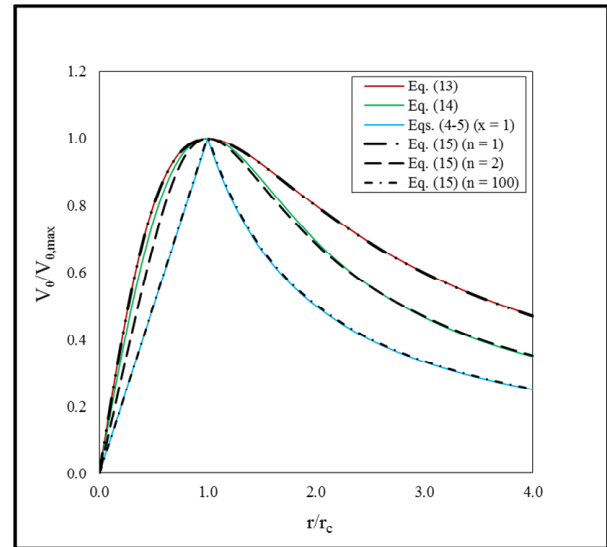
Both algebraic TVPs accurately approximate the derived TVPs. The W-W profile provides slightly better approximation of the L-O profile for $r < r_c$, but Vatistas' profile better approximates both the L-O and S-K profiles for $r > r_c$. Moving forward, Vatistas' approximations of the derived TVPs shall be considered save two exceptions. The Sullivan vortex shall be represented by the W-W approximation. Also, equations (4-5) shall be used for the MRCVM for $x \neq 1$.

Omitted Measured Tangential Velocity Profiles

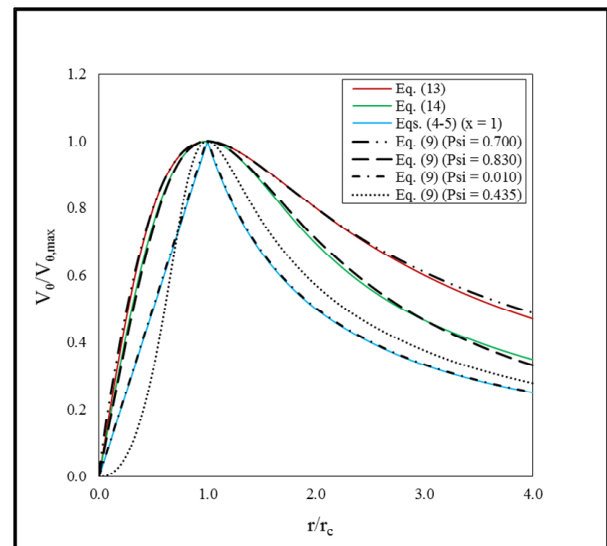
Several measured TVPs are excluded from the comparison because they outlie the majority of the

other collected TVPs within their categories. It is therefore believed that the measurements were somehow flawed.

From the vortex chamber experiments, the data of Faler and Leibovich (1977) is omitted. Their measured TVP shows unrealistically-rapid decay for $r/r_c > 2.5$. It is likely that they report measurements taken too closely to the wall of their experimental system, hence the confining walls force the decay of the vortex.



(a)



(b)

Figure 9. Comparison of derived analytical TVPs with approximations by (a) Vatistas and (b) Wood and White's TVPs.

Selection of a Realistic Viscous Vortex Tangential Velocity Profile

From the tornado measurements, the data of Hoecker (1960) and Golden (1974) is omitted. There measured TVPs are derived by tracking debris movement between successive photographs; this procedure gives at best qualitative results which deviate substantially for $r/r_c > 1$ from the more recent radar measurements.

Lastly and also from the tornado measurements, the data of Bluestein et al. (2003) are omitted. Their measurements are conducted using the same radar technology and similar measurement distances as the other radar data sources. However, unrealistically-rapid decay of the TVP occurs for $r/r_c > 1.5$.

Comparison of Measured and Analytical Profiles

The measured TVPs are compiled and compared with the analytical TVPs in Figures 10a and 10b. The six defined groups of measured TVPs are plotted as two data sets to avoid excessive overlap and saturation of the data.

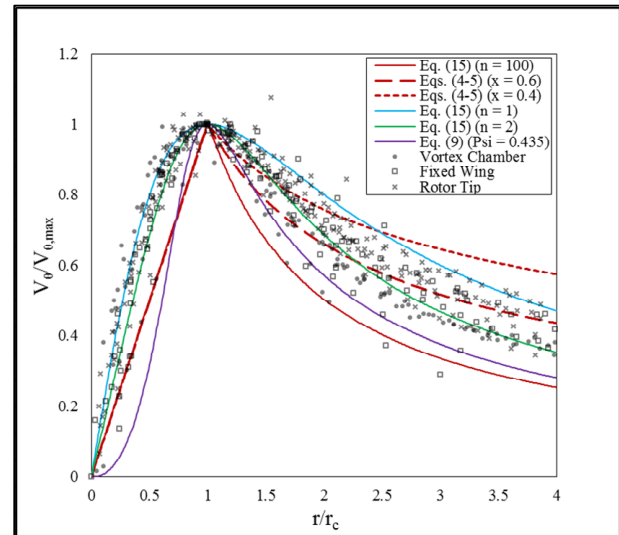
Beginning with Figure 10a, Vatistas' $n = 1$ and $n = 2$ profiles are excellent fits to the measured vortex chamber, fixed wing, and rotor tip TVPs for $r/r_c \leq 1$. For $r/r_c > 1$, the $n = 1$ profile is effectively an upper boundary for the measured TVPs. The $n = 2$ profile falls from the middle of the measured profiles at $r/r_c > 1$ to effectively become a lower boundary for the measured TVPs at $r/r_c = 4$. The MRCVM and W-W (Sullivan) profiles deviate substantially from the measured TVPs for $r/r_c < 1$. The MRCVM $x = 0.6$ profile gives a fair approximation of the measured TVPs for $r/r_c > 1.5$, but the MRCVM $x = 1.0$ and $x = 0.4$ models consistently under- and over-estimate the measured TVPs respectively.

Now moving to Figure 10b, W-W's Sullivan profile is an effective lower boundary to the simulated tornado, tornado, and hurricane TVPs. Vatistas' $n = 1$ profile is an effective upper boundary for $r/r_c \leq 1.5$, but falls within the measured hurricane TVPs when $r/r_c > 1.5$. The MRCVM $x = 0.6$ profile provides a higher upper bound for $r/r_c > 2.5$, but is a poor fit to the measured TVPs for $0.5 \leq r/r_c \leq 1.5$.

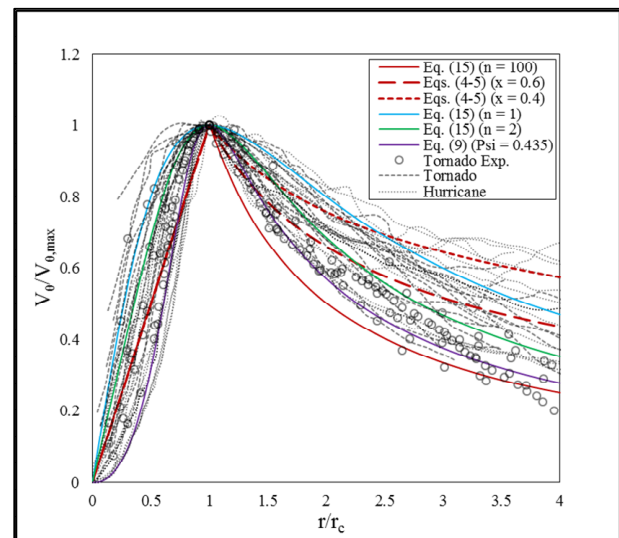
Summary and Conclusions

Realistic computer simulation of vortex-loading of structures necessitates the use of a realistic vortex tangential velocity profile (TVP). The physics that govern vortex structure are not well understood, hence the best approach for selecting a realistic vortex TVP for integration in a computer model is to find the analytical TVP which best represents measured TVPs

in experimental and naturally-occurring vortices.



(a)



(b)

Figure 10. Comparison of measured and analytical TVPs.

An extensive survey of measured TVPs is conducted, and TVPs from various experimentally-produced vortices as well as tornado and hurricane vortices are compiled. Subsequently, analytical TVPs are compiled and normalized for comparison with the measured vortex TVPs. Based upon the content presented in this work, the conclusions outlined below have been reached.

1. Vatistas $n = 1$ analytical profile (the

normalized S-K vortex) is an effective upper boundary to most of the measured TVPs.

2. Vatistas $n = 2$ analytical profile (the normalized L-O/B-R vortex) bisects most of the measured TVPs, hence it represents the “typical” vortex.

3. The W-W (Sullivan) analytical profile provides the best lower boundary to the data. It is an excellent lower boundary for the following categories of measured TVPs: experimental tornado, measured tornado, and measured hurricane. However, it deviates greatly from the measured vortex chamber, fixed wing, and rotor time vortex TVPs for $r < r_c$.

4. The analytical MRCVM profile deviates greatly from the measured TVPs for $0.75 \leq r/r_c \leq 1.25$ due to the peaked profile near $r = r_c$. The MRCVM with $x = 0.6$ fits the measured TVPs for $r/r_c > 1.25$.

Vatistas' $n = 2$ profile best approximates the typical, measured TVP, hence it shall be applied in subsequent computer simulations. It is stressed, however, that no single TVP defines all vortices. Therefore, studies need to be conducted to evaluate the influence of the vortex's TVP on structure loading that it produces.

Future Work

A range of possible vortex TVPs has been identified. Although the “typical” vortex profile has been suggested for general computer simulations, it is necessary to determine the influence of vortex profile on structure loading. Computer modeling is currently being used to study the influence of vortex TVP on maximum structure loading and dynamic amplification of structure loading.

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A Case of Frugivory in a Green Treefrog (*Hyla cinerea*) from Northeastern Arkansas

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Running Title: Frugivory in a Green Treefrog

Abstract

An adult green treefrog (*Hyla cinerea*) was collected in mid-September 2014 from Jonesboro, Craighead County, Arkansas. Contents included the remains of a beetle and two fruits. The contents were photographed, measured, and identified to the lowest achievable taxonomic level. The beetle was identified as a spotted cucumber beetle (*Diabrotica undecimpuncta howardi* Barber [Coleoptera: Chrysomelidae]). The fruits were identified as *Persicaria* sp. (likely *P. punctata* (Elliot) Small; Polygonaceae) and *Oryza sativa* L. (Poaceae). Fruits in the stomachs of frogs are rarely reported in the literature, but could represent possible mechanisms for seed dispersal in plants. It is unknown if frogs select to sometimes eat fruits or if fruits are a by-product of animal prey capture or missed predation attempts. In any case, the goal of this report is to raise awareness of a poorly documented phenomenon in an effort to direct attention to this possible method of seed dispersal.

Introduction

Amphibians are generally opportunistic carnivores and will eat anything they can swallow (Duellman and Trueb 1986, Stebbins and Cohen 1995). In most cases, visual stimuli induce a feeding response, but not much is known about prey selection and foraging strategies (Duellman and Trueb 1986, Stebbins and Cohen 1995). When pursuing prey, an amphibian may incidentally ingest non-prey material such as sediment or plant matter (Korschgen and Moyle 1955, Linzey 1967, Hedeon 1972, Hirai and Matsui 1999, Santos et al. 2004).

Movement may also elicit ingestion of non-prey material. Frogs and toads have been found to ingest fishing lures (Stebbins and Cohen 1995), stones (Engelbert et al. 2008), and plant matter, such as seeds or flowers, if perceived as prey (Hamilton 1948, Oliver 1955, Stebbins and Cohen 1995). In most cases, it is unclear if ingestion of plant material is deliberate (but

see Silva et al. 1989), but selecting for vegetation in the diet may provide anurans benefit (Anderson et al. 1999). Therefore, fruit-eating may prove to be a possible method of seed dispersal (Silva et al. 1989, Fialho 1990).

Herein, we present an observation of frugivory (i.e., fruit-eating) by a green treefrog (*Hyla cinerea*) from northeastern Arkansas. This report describes this incident and is intended to encourage further studies of amphibians ingesting plant material and potentially acting as seed dispersers.

Materials and Methods

An adult green treefrog was collected in Jonesboro, Craighead County, Arkansas, during mid-September 2014. The frog was euthanized in a dilute chlorobutanol solution before being measured and examined. A hard mass was noticed during the examination and dissection was initiated. The stomach was visibly full and was removed. The frog was fixed in 10% neutral buffered formalin for 48 hours before being transferred to 70% v/v ethanol and deposited into the ASU Herpetological Museum. The contents of the stomach were removed and placed into a vial. Both the stomach and contents were fixed in 10% neutral buffered formalin for 48 hours before being transferred to 70% v/v ethanol. Stomach contents were photographed and identified to lowest achievable taxonomic level.

Results

Stomach contents included the elytra of a spotted cucumber beetle (*Diabrotica undecimpuncta howardi* Barber [Coleoptera: Chrysomelidae]) and two fruits identified as *Persicaria* sp. (likely *P. punctata* (Elliot) Small; Polygonaceae), commonly known as dotted knotweed, and *Oryza sativa* L. (Poaceae), commonly known as Asian rice. The treefrog measured 27 mm snout-vent length (Figure 1A), and its emptied stomach measured 10 mm long by 4 mm wide (Figure 1B-C).

Frugivory in a Green Treefrog

The fruit of the Asian rice measured 8 mm long by 3 mm wide (Figure 1D), and the *Persicaria* fruit measured 3 mm long by 2 mm wide (Figure 1E-F).



Figure 1: Photographs of treefrog (A), stomach contents (B width and C length), *Oryza sativa* L. fruit (D), and *Persicaria* sp. fruit (E with persistent perianth and F with perianth peeled away from achene). Arrows point to beetle elytra (B), *O. sativa* L. fruit (g) and treefrog stomach (s), and the perianth (E) and achene fruit (F) of *Persicaria* sp. Ruler lines denote millimeters.

Discussion

Although this is a solitary case of frugivory, further studies may determine if fruit-eating is accidental or deliberate and how prevalent it is in treefrogs. The presence of two fruits of different plants, however, may indicate potential cases of frugivory are fairly common. The size of the *O. sativa* L. fruit is also of interest. The relaxed stomach measured only two mm longer and one mm wider than the fruit. We believe that the frog may not have been able to pass this fruit naturally, which could result in a forcible expulsion through regurgitation, or impaction, which could lead to death. The *Persicaria* sp. fruit, however, was small enough to be easily passed.

Interestingly, both fruits appeared to be undigested, while the only remnants of the beetle were the elytra. If the stomach acid does not damage the fruit, and it is passed or regurgitated, it may germinate, as seen by Fialho (1990) in *Xenohyla truncata*. If fruits eaten by treefrogs can germinate, then the treefrogs may act as a dispersal agent for the plant species.

Acknowledgments

Authorization of treefrog collection was granted by a collection permit from the Arkansas Game and Fish Commission. We would like to thank Johnny Konvalina for specimen collection. We also thank Dr. Gregory Phillips for use of his photomicroscope. The treefrog was deposited in the Arkansas State University Herpetological collection under ASUMZ 33290.

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Testicular Histology and Sperm Morphometrics of the Bird-voiced Treefrog, *Hyla avivoca* (Anura: Hylidae), from Arkansas

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Running Title: Testicular Histology and Sperm Morphometrics in *Hyla avivoca*

Abstract

We examined the testicular histology and spermatozoal dimensions of the bird-voiced treefrog, *Hyla avivoca* (Anura: Hylidae), from samples collected in May, June, and July from localities in three counties (Calhoun, Conway, and Little River) in Arkansas. Calling frogs were necropsied in the lab, and testes were prepared for light and scanning electron microscopy. Spermatocysts within seminiferous tubules of all males contained large aggregates of spermatozoa. Primary spermatogonia, the largest of all germ cells, ranged from 13.3 – 17.8 μm in diameter ($\bar{x} = 15.37 \pm 1.22$; $n = 20$). Measurements of sperm dimensions yielded the following length parameters (range, mean \pm standard deviation, sample size): acrosome, 2.10 – 3.37 μm ($\bar{x} = 2.58 \pm 0.40$; $n = 11$); nucleus, 10.22 – 13.71 μm ($\bar{x} = 11.70 \pm 0.86$; $n = 65$); acrosome, nucleus, midpiece complex (ANM) in three frogs, 14.87 – 23.98 μm ($\bar{x} = 19.62 \pm 2.72$; $n = 17$), 18.83 – 26.96 μm ($\bar{x} = 22.92 \pm 2.26$; $n = 17$), 17.40 – 26.96 μm ($\bar{x} = 23.92 \pm 3.27$; $n = 11$); principal piece, 24.36 – 27.68 μm ($\bar{x} = 25.98 \pm 1.19$; $n = 14$); total tail length (endpiece intact), 29.87 – 39.00 μm ($\bar{x} = 33.37 \pm 2.63$; $n = 23$); and total sperm length, 51.02 – 62.98 μm ($\bar{x} = 54.63 \pm 3.54$; $n = 20$). Our sperm morphometric findings complement previously published data on this species and fill in gaps that may aid in future intra- and interfamilial comparisons.

Introduction

The bird-voiced treefrog, *Hyla avivoca*, is a small hyliid species that is found primarily in cypress/tupelo swamps, swampy floodplains, and swampy rivers, lakes and ponds in the southeastern and southcentral United States (Dodd 2013). The species' range extends northward into extreme southern Illinois, southeastern Missouri, and western portions of Kentucky and Tennessee. Western populations are scattered in parts

of the Arkansas Valley and Ouachita River basin in Arkansas (Fulmer and Tumlison 2004; Trauth et al. 2004). The biology of this species was recently summarized by Dodd (2013).

The sperm morphology/ultrastructure of relatively few species of the 40 or so genera of hyline frogs (Vitt and Caldwell 2014) has been investigated (Scheltinga et al. 2002; Scheltinga and Jamieson 2003). For example, other than a light microscopic analysis by Delahoussaye (1966), who reported on the sperm structure in 10 hyliid species from Louisiana (including *Hyla avivoca*), only one other North American hyliid, *Pseudacris regilla*, has received any detailed attention regarding sperm structure (Scheltinga 2002). Moreover, Delahoussaye (1966) did not provide any descriptive accounts of testicular microanatomy on any of the species he examined and, more importantly, he was unable to measure several dimensions of sperm morphology in most species due to the limitations in his light micrographic techniques.

In the present study, we report on the testicular histology of the bird-voiced treefrog, *Hyla avivoca*, and provide additional morphometric information on sperm structure obtained using scanning electron microscopy that is not available in the study by Delahoussaye (1966).

Materials and Methods

Calling frogs were collected in several counties (Calhoun—7 May 2013 [$n = 1$], 22 July 2014 [$n = 4$]; Conway—9 June 1991 [$n = 17$], and Little River—18 June 1997 [$n = 8$]) of central and southern Arkansas. Live specimens were returned to the Electron Microscope Facility at Arkansas State University (ASU) and processed for histological analyses. The frogs were sacrificed by submersion into a dilute chlorotone solution, and the testes prepared for both light and scanning electron microscopy (LM and SEM, respectively). Snout-vent length (SVL) was measured

to the nearest mm. Voucher specimens of the frogs were deposited in the ASU herpetological collection (ASUMZ), whereas reproductive tissues embedded in plastic resin were stored in the histo-herpetological collection of the senior author.

For LM, we fixed plastic-embedded testes in 2% glutaraldehyde (GTA). Testes were then dehydrated in a graded series of increasing ethanol solutions (50-100%), placed in a 50/50% acetone/plastic mixture for overnight infiltration, and embedded in Mollenhauer's Epon-Araldite #2 (Dawes 1988). For thick sectioning (approximately 1 μ m in thickness) and staining, we used glass knives on an LKB Ultratome (Type 4801A) with Ladd® multiple stain (LMS), respectively. For photomicroscopy, we utilized a Nikon Eclipse 600 epifluorescent light microscope with a Nikon DXM 1200C digital camera (Nikon Instruments Inc, Melville, NY).

For SEM, we fixed sperm samples on cover slips (18 mm X 4 mm) in a 2% GTA solution buffered with 0.1 M sodium cacodylate at a pH of 7.2 for a minimum of 2 h. The cover slips, previously coated with Poly-L-lysine, were dehydrated in a graded series of increasing ethanol solutions (50-100%), followed by several fluid exchanges in 100% ethanol. An Autosamdri-815 critical point drier (Tousimis Research Corporation, Rockville, MD) was used (31°C, 1072 psi, ventilation rate ~100 psi/min) to remove excess ethanol. Cover slips were adhered to copper boats and then mounted on 25.4 mm aluminum pin stub specimen mounts and coated with gold using a Cressington 108 sputter coater (Cressington Scientific Instruments Ltd, Watford, UK). Sperm samples were analyzed both qualitatively and quantitatively with a Vega TS 5136XM digital scanning electron microscope (Tescan USA Inc., Cranberry Township, PA) at 19.5 kV.

For the descriptive terminology of testicular histology, we followed Rastogi et al. (1988), Pudney (1995) and Scheltinga and Jamieson (2003). For describing the sperm ultrastructure, we followed Scheltinga and Jamieson (2003). Measurements of sperm structure for both LM and SEM images were obtained using ImageJ software (National Institutes of Health). Descriptive statistics included means \pm 1 standard deviation.

Results

Testicular Histology and Spermatogenesis

As with all anamniotes, the testes of *Hyla avivoca* exhibit cystic spermatogenesis. The testes are organized into numerous seminiferous tubules, whose

seminiferous epithelia possess capsular-like follicles, called spermatocysts. Numerous Leydig cells (Fig. 1) are situated within the interstitial spaces along the periphery of each tubule. There can be as many as 20-25 spermatocysts observed in any one cross-sectional histo-section through a single tubule (Fig. 1). In addition, the spermatocysts appear to be aligned with one another in one or more layers extending away from the basement membrane (Figs. 1B and 2). Spermatocysts originate from a single large primary spermatogonium (Fig. 2 A and B), also called a primordial germ cell, which undergoes several mitotic

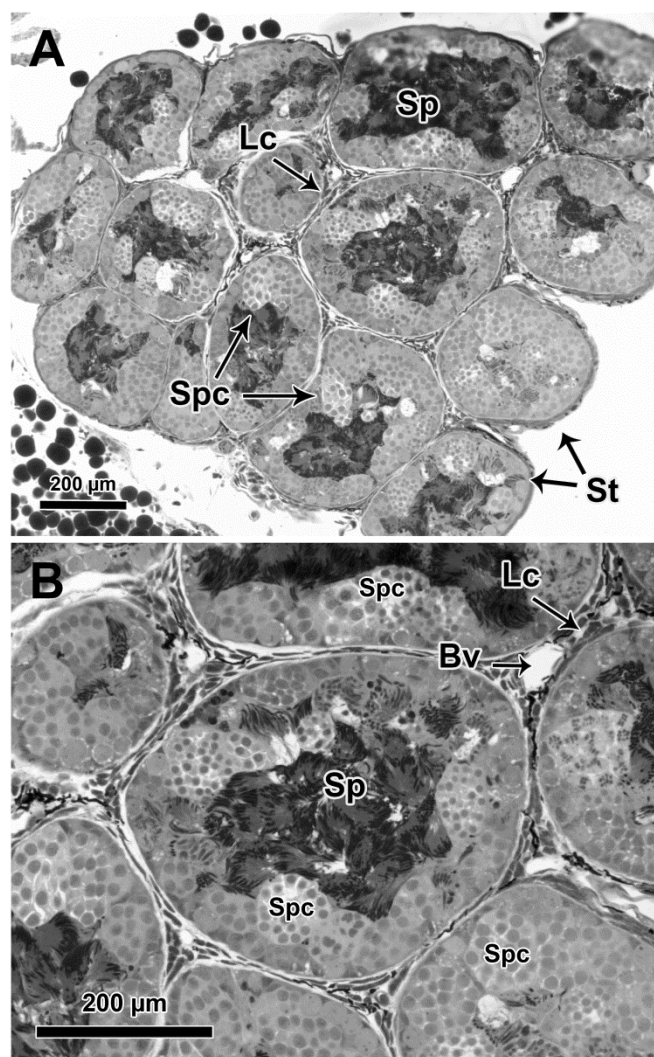


Figure 1. Light micrographs of the seminiferous tubules of a plastic-embedded testis of *Hyla avivoca*. A. Transverse section through a testis from a late July specimen (ASUMZ 33250) revealing sperm (Sp) aggregates within most lumina of seminiferous tubules (St); Lc = Leydig cells. Individual spermatocysts (Spc), which line the basement membrane are also evident. B. Magnification of A showing spermatocysts in different cell stages of spermatogenesis. Bv = blood vessel.

Testicular Histology and Sperm Morphometrics in *Hyla avivoca*

divisions to eventually yield clonal aggregates of germ cells (secondary spermatogonia to mature spermatozoa) through the process of spermatocytogenesis. Primary spermatogonia, the largest of all germ cells, ranged from 13.3 – 17.8 μm in diameter ($\bar{x} = 15.37 \pm 1.22$; $n = 20$).

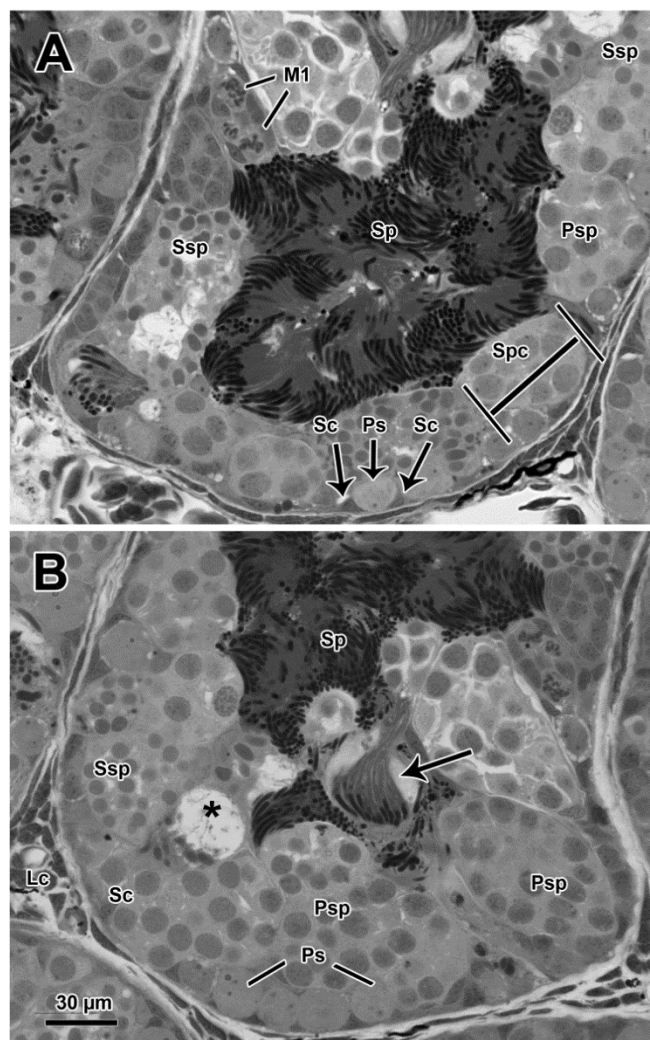


Figure 2. Light micrographs of the seminiferous tubules of a plastic-embedded testis of *Hyla avivoca* (from section in Fig. 1A). A. Transverse section through a portion of a seminiferous tubule showing spermatocysts (Spc) with germ cell clusters in different stages of development including primary spermatocytes (Psp) and secondary spermatocytes (Ssp). Mature sperm (Sp) dominate the tubular lumen. Nuclei of Sertoli cells (Sc) are positioned basally between primary spermatogonia (Ps) along the basement membrane. Spermatocytes of meiotic cells (M1) are also shown. B. Adjacent portion of tubule shown in A showing additional spermatocysts. Interstitial Leydig cells (Lc) can be seen bordering tubule at lower left. A cluster of mature sperm (arrow) is seen exiting a spermatocyst. Cellular debris resides in a spermatocyst devoid of sperm (asterisk). Abbreviations are the same as in A. Scale bar the same for A.

As testicular activity continues, spermatocysts rapidly increase in size and produce a variety of spermatogenic cell stages (Fig. 2). Most testes measured 3 – 4 mm in length. Metaphase of mitotic divisions of secondary spermatogonia within a spermatocyst is shown in Fig. 2B. Several of these spermatogenic stages (e.g., primary spermatocytes, secondary spermatocytes, and spermatids) are also shown in Fig. 2. The nuclei of Sertoli cells (= sustentacular cells) are prevalent along the basement membrane as they flank each primary spermatogonium (Fig. 2); on the other hand, the cytoplasm of Sertoli cells may extend well into the lumen of each tubule. Clonal clusters of sperm remain together as they exit rupturing spermatocysts (Fig. 2B).

Sperm Morphometrics

The morphology of a mature hyloid spermatozoon consists of an acrosome, nucleus, midpiece, and tail (principal piece and endpiece) and was illustrated most recently by Scheltinga and Jamieson (2003). In the present study, measurements of the lengths of the nucleus (Fig. 3) were accomplished using LM, whereas lengths of individual acrosomes, the acrosome, nucleus and midpiece complex (ANM) as well as the principal piece, endpiece, and total sperm length were best achieved using SEM (Fig. 4).

The acrosome ranged from 2.10 – 3.37 μm in length ($\bar{x} = 2.58 \pm 0.40$; $n = 11$), whereas the nucleus ranged from 10.22 – 13.71 μm in length ($\bar{x} = 11.70 \pm 0.86$; $n = 65$). The ANM complex varied somewhat among three males collected on 7 May 2013. For example, in ASUMZ 32704 (SVL = 37 mm), the ANM ranged from 14.87 – 23.98 μm in length ($\bar{x} = 19.62 \pm 2.72$; $n = 17$). In ASUMZ 32705 (SVL = 38 mm), ANM ranged from 18.83 – 26.96 μm in length ($\bar{x} = 22.92 \pm 2.26$; $n = 17$), and lastly ASUMZ 32706 (SVL = 40 mm), the ANM ranged from 17.40 – 26.96 μm in length ($\bar{x} = 23.92 \pm 3.27$; $n = 11$).

Tail length measurements pose a problem in hyloid frogs due to the fact that the endpiece is fragile (Delahoussaye 1966). During the preparatory process for SEM in the present study, many of the sperm tails examined did not retain their endpieces. Consequently, tail length measurements consisted of two sets of values: one was considered principal piece only, whereas the other was total tail length (endpiece intact). The former ranged from 24.36 – 27.68 μm in length ($\bar{x} = 25.98 \pm 1.19$; $n = 14$), and the latter value ranged from 29.87 – 39.00 μm in length ($\bar{x} = 33.37 \pm 2.63$; $n = 23$). By noting the size difference between total tail length and principal piece length, a rough

estimate of the endpiece length is around 8 μm . Total sperm length was calculated using only sperm that appeared to possess an intact endpiece; these data yielded a range of 51.02 – 62.98 μm in length (\bar{x} = 54.63 \pm 3.54; n = 20).

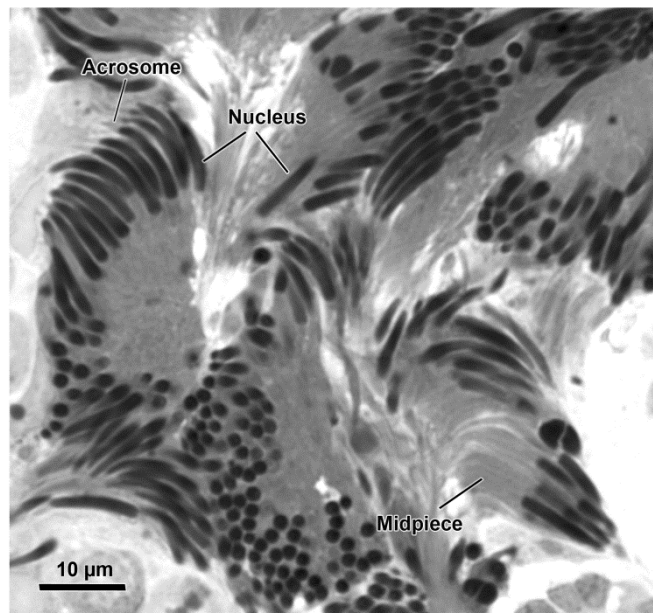


Figure 3. Light micrograph within the central luminal region of a seminiferous tubule showing clonal clusters of mature sperm. The acrosome, nucleus, and midpiece are easily discernible.

Discussion

Mature sperm dominated the luminal interior of seminiferous tubules in all frogs examined during this study. It has been estimated that there are 7 – 8 divisions occurring from each primary spermatogonium in some frog species (Rastogi et al. 1988, Takamure et al. 1995), which yield large numbers (2^8) of primary spermatocytes.

Morphometric comparisons between our sperm data and those reported by Delahoussaye (1966) revealed several differences. He found a smaller average acrosome length ($2.1 \mu\text{m} \pm 0.2$; range, 1.9 – 2.4), and his average ANM ($18.6 \mu\text{m} \pm 1.1$; range, 17.1 – 20.6) was also smaller than our calculations on three males. Moreover, he was unable to accurately determine tail length and, therefore, reported no total sperm length values. Our results suggest that total sperm length for most sperm will fall within 50 – 60 μm . Scheltinga (2002) reported a total sperm length in *Pseudacris regilla* to be 50 μm . Of the 36 species of Australian hyloid frogs examined by Scheltinga (2002),

16 (44.4%) exhibited total sperm lengths within the 50 – 60 μm range.

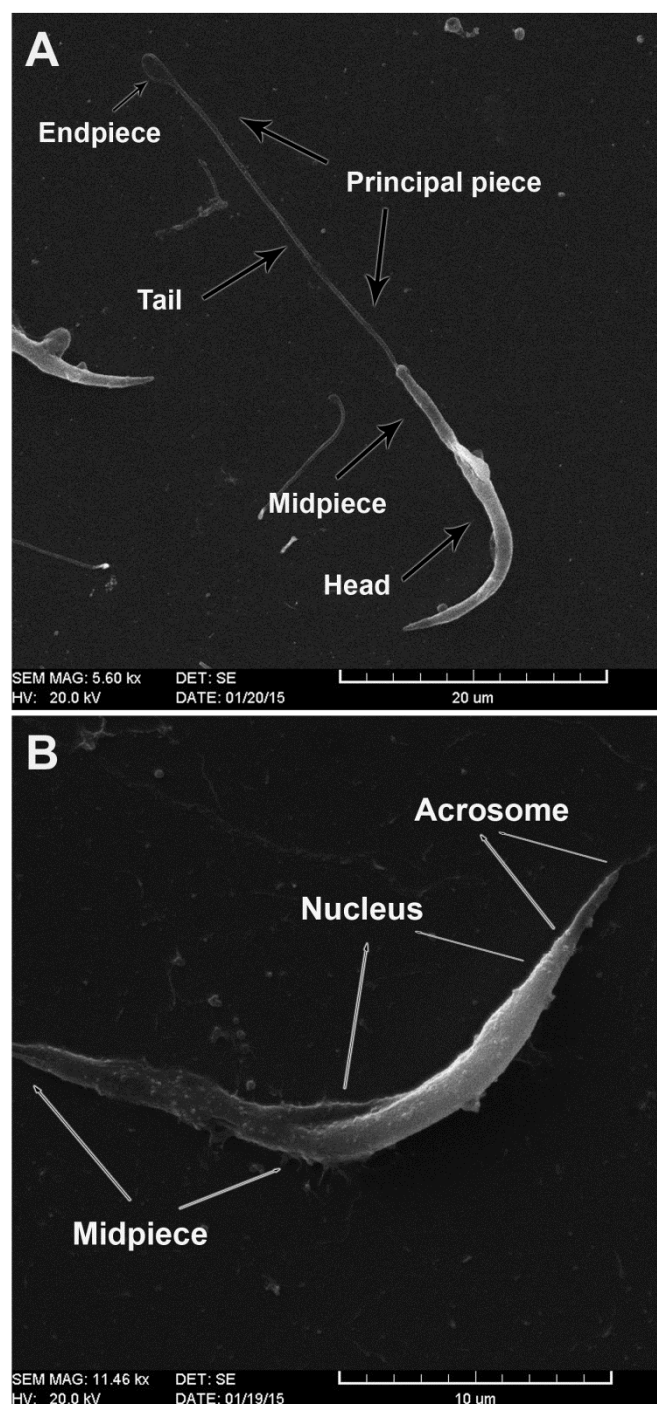


Figure 4. Scanning electron micrographs of mature sperm of *Hyla avivoca* (ASUMZ 32706). A. Spermatozoon (total length = 60.84 μm) showing anatomical regions including the head, midpiece, and tail (principal piece and endpiece). B. The acrosome, nucleus, and midpiece complex and their distinct morphologies.

Testicular Histology and Sperm Morphometrics in *Hyla avivoca*

Future studies on anuran sperm morphometrics will benefit greatly by incorporating SEM analyses into their techniques. This will allow for useful comparisons to be made for a better understanding of sperm structure.

Acknowledgments

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Vertebrate Natural History Notes from Arkansas, 2015

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Abstract

Many important details of vertebrate biology are unknown to the scientific community because the observations are not part of a larger theoretical study. Yet, knowledge of such details not only fills gaps in understanding but also creates a framework for hypothesis building. We have collected observations of importance that can add to the growth of knowledge. Compiled here are important recent observations representing all vertebrate classes in Arkansas.

Introduction

Although vertebrates are a commonly studied group of animals, the distribution and natural history of many species within Arkansas is still not well understood or documented. We have been developing a series of articles to update the state of knowledge of the natural history of Arkansas's vertebrates (e.g. Tumilson et al. 1992, Tumilson and Robison 2010, Connior et al. 2011, 2012, 2013, 2014b). We augment current literature with new records of distribution and provide notes on the natural history of selected vertebrates from Arkansas. Herein we include previously unreported records of distribution, reproduction, parasites, and other aspects of natural history of the vertebrates of Arkansas.

Methods

We collected fishes by use of 3.1×1.8 m or 6.1×1.8 m seines with 3.2 mm mesh. Specimens of fishes, amphibians and reptiles were preserved in 10% formalin and stored in 45% isopropanol. We report localities as section (Sec.) township (T), and range (R),

or as latitude and longitude values.

Bat records are based on identification of specimens sent to the Arkansas Department of Health for rabies testing or from field records of mist-netting activities (Arkansas Game and Fish Commission [AGFC] cave bat database records and AGFC mist netting operations), with release of captured animals on site following data collection. Observers names (initials if one of the authors) are provided in select accounts.

All vertebrate voucher specimens (physical or photographic) are deposited in the Southern Arkansas University Vertebrate Collection (SAU) in Magnolia or the vertebrate collections at Henderson State University (HSU). Voucher ectoparasite specimens are deposited in the General Ectoparasite Collection in the Department of Biology at Georgia Southern University.

Results and Discussion

CLASS ACTINOPTERYGII

Cyprinidae – Carps and Minnows

Cyprinella galactura (Cope) – Whitetail Shiner. Five specimens of *C. galactura* were collected by HWR and CTM on 23 July 2014 from Crooked Creek at Kelly's Slab, ca. 12.9 km W of Yellville, Marion Co. (Sec. 6, T18N, R16W), including 2 tuberculate males and 3 gravid females. Pflieger (1997) reported that this shiner spawns from early June to mid-August in Missouri. In Arkansas we have little information on breeding times of this cyprinid. The discovery of these breeding specimens infers a similar spawning period for *C. galactura* in Arkansas.

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Hybopsis amblops (Rafinesque) – Bigeye Chub. McAllister et al. (2009, 2010) reported a new occurrence of the Pallid Shiner, *Hybopsis amnis*, from the lower Strawberry River, Lawrence Co., Arkansas. However, subsequent examinations by one of the authors of the original paper (WCS) revealed the single specimen (North Carolina State Museum 47146) to be *Hybopsis amblops*, a species previously known from the Strawberry River system (Robison and Buchanan 1988), though most records are from more upland portions of the system.

Notropis sabinae Jordan and Gilbert – Sabine Shiner. Green and Beadles (1974) surveyed the fishes of the Current River, but did not report *N. sabinae*. Two specimens of *N. sabinae* were taken on 4 April 2014 from the Current River over a sandbar at Cox Road NW of Biggers, Randolph Co. (Sec. 26, T20N, R2E). These specimens represent the first documented record of the uncommon Sabine Shiner from the Current River system (Robison and Buchanan 1988).

Opsopoeodus emilae Hay – Pugnose Shiner. This common lowland shiner was not listed by Green and Beadles (1974) in their study of the fishes of the Current River in Arkansas. On 4 April 2014 a single specimen of *O. emilae* was collected over a sandbar on the Current River at Cox Road NW of Biggers, Randolph Co. (Sec. 26, T20N, R2E). Because the river was high and turbid at the time from recent rains, this specimen may represent a waif from a nearby tributary stream. This specimen is the first documented record of the Pugnose Shiner from the Current River system (Robison and Buchanan 1988).

Pimephales tenellus (Girard) – Slim Minnow. Robison and Buchanan (1988) mapped the statewide distribution of the Slim Minnow. Two specimens of this rarely encountered cyprinid were collected from the Spring River at Imboden, Lawrence Co. (Sec. 15, T18N, R2W) on 16 June 1999 by HWR. Previous surveys had not detected this species in the Spring River system (Winters, 1985; Robison and Buchanan, 1988), so this is the first report of its occurrence.

Pteronotropis hubbsi (Bailey and Robison) – Bluehead Shiner. On 5 July 1996, RT collected this species at 3 locations in Ashley Co., which represents a new county record (Robison and Buchanan 1988). Two locations were near the Saline River: at a slough off of Saline River, 14.5 km (ca. 9 mi.) NW of Crossett, Sec. 4, T17S, R9W; and Thompson Creek, 11.3 km (7 mi.)

NW of Crossett, Sec. 11, T17S, R9W. On 11 April 1997, specimens also were taken from the Ouachita River 10.5 km (6.5 mi.) W of Crossett off U.S. Hwy 82, Sec. 14, T18S, R10W. The AGFC currently treats this fish as a Species of Special Concern.

Esocidae – Pikes and Mudminnows

Esox niger Lesueur – Chain Pickerel. Robison and Buchanan (1988) stated the Chain Pickerel was most abundant in streams and lakes of the Coastal Plain lowlands in southern and eastern Arkansas, although it did penetrate somewhat above the Fall Line in streams of the Ouachita Mountains and to a lesser extent in streams above the Ozark Mountains boundary. They also noted this species was virtually absent from most of the Ozarks and the upper Arkansas River drainage. We report 2 new records of *E. niger* in northern Arkansas. The first is a single specimen (363.2 mm TL) collected from the lower Strawberry River at AR St. Hwy 361 in Lawrence Co. ca. 3.2 km E of Saffell (Sec. 30, T15N, R2W) on 20 June 1996. The discovery of *E. niger* is noteworthy as it is the first documented occurrence of this species from the Strawberry River system (Robison and Harp 1971, Robison and Beadles 1974) and brings to 110 the number of fish species recorded from the Strawberry River system, making it one of the richest river systems in the central United States. The second recent record in NE Arkansas is of a single specimen taken on 21 April 2014 using a "Missouri Trawl" in the Little River drainage (35.997797°N, -90.090248°W), Mississippi Co. (Robert Hrabik, *pers. comm.*).

Ictaluridae – Catfishes

Noturus eleutherus Jordan – Mountain Madtom. Six adult (46-52 mm TL) Mountain Madtoms were collected from the Little Missouri River at the Nubbin Hill Access, Nevada Co. on 27 November 2014 by CTM using a backpack electroshocker. This represents the first record of *N. eleutherus* from this upstream region of the Little Missouri River as previous records are 26 km to the southeast near the northern end of White Oak Lake (Robison and Buchanan 1988). Habitat consisted of rock and gravelly substrate without aquatic vegetation in midstream (depth = 0.9 m) over a moderately flowing river without riffles.

Aphredoderidae – Pirate Perch

Aphredoderus sayanus (Gilliams) – Pirate Perch. We report 2 new populations of *Aphredoderus sayanus*. Pirate Perch are typically lowland fishes (Robison and Buchanan 1988), thus its discovery in an

upland habitat is surprising. On 5 April 2014, 6 specimens were collected from a dense population of *A. sayanus* in a large spring at Spring Mill off AR St. Hwy 69 in James Switch, Independence Co. (Sec. 27, T14N, R7W). An additional specimen running mill was captured on 6 April 2014 off AR St. Hwy 31 in Mill Creek at Floyd Baptist Church, White Co.

Fundulidae – Topminnows

Fundulus catenatus (Storer) – Northern Studfish. *Fundulus catenatus* is an upland topminnow that typically feeds on insects and small molluscs (Robison and Buchanan 1988). Few observations are available of the foods of this topminnow in Arkansas. On 23 July 2014, an adult female *F. catenatus* (110 mm TL) was captured by HWR and CTM from Crooked Creek at Kelly's Slab, ca. 12.9 km W of Yellville, Marion Co. (Sec. 6, T18N, R16W). The stomach contained a 45 mm TL Duskystripe Shiner, *Luxilus pilsbryi*. This appears to be the first report of *F. catenatus* feeding on another fish. A single crayfish (*Orconectes* sp.) was found in stomachs of each of 2 additional *F. catenatus* from the same site. One specimen of a terrestrial hemipteran, *Melanolestes picipes* Herrich-Schaeffer (Family Reduviidae), was in one of the stomachs. The female *F. catenatus* was full of ripe eggs indicating imminent spawning.

Fundulus chrysotus (Günther) – Golden Topminnow. The Golden Topminnow is not collected commonly in southern Arkansas. We report 3 new populations of this lowland species. Two *F. chrysotus* were taken on 7 April 2014 off AR St. Hwy 11 from Long Lake (an oxbow) at Woodville, Lincoln Co. (Sec. 10, T7S, R6W). Five additional specimens of *F. chrysotus* were collected on 27 June 2014 from Cane Creek Lake at the boat ramp, Lincoln Co. (33.916525°N, -91.76517°W). On 29 June 2014, 5 *F. chrysotus* were taken from Silver Lake (an old oxbow off Arkansas River) at AR St. Hwy 212, NE of Dumas, Desha Co. (Sec. 30, T8S, R3W).

PERCIDAE – True Perches

Ammocrypta clara Jordan and Meek – Western Sand Darter. Green and Beadles (1974) surveyed the fishes of the Current River, but did not list *A. clara* among the fishes. A single specimen of this uncommon darter was captured on 4 April 2014 from the Current River at a sandbar off Cox Road NW of Biggers, Randolph Co. (Sec. 26, T20N, R2E). This is the first documented occurrence of *A. clara* occurring in the Current River system (Robison and Buchanan

1988).

Etheostoma artesiae (Hay) – Redspot Darter. The Redspot Darter was elevated from subspecies designation (*E. whipplei artesiae*) by Piller et al. (2001). This lowland darter is a rather uncommon percid in southern Arkansas, inhabiting streams of the Coastal Plain. On 15 September 1995, a single specimen was captured by HWR and SAU students from Bayou Bodcau at a gravel road bridge 8.0 km N of Stamps, Lafayette Co. (Sec. 17, T15S, R24W). This specimen is the first record of this darter from the Bayou Bodcau drainage system in southern Arkansas.

CLASS AMPHIBIA

Ranidae – True Frogs

Lithobates areolatus circulosus (Rice and Davis) – Northern Crawfish Frog. The Northern Crawfish Frog inhabits extreme northwestern Arkansas through the Arkansas River Valley and to the southeastern corner of the state (Trauth et al. 2004). Knowledge regarding its ecology and reproduction are limited due to its secretive habits. In southeastern Arkansas (the campus of UAM in Drew Co.), males arrived at the breeding areas as early as January after rains, and females and amplexant pairs were observed on 23 February (Bacon and Anderson 1976). At that location, the height of the breeding season was during February though a few individuals called through March (Bacon and Anderson 1976). Trauth et al. (1990) reported calling males from late February to early April, but did not disclose any locality information. We report on breeding biology in northwestern Arkansas during late winter 2015. MBC discovered several breeding areas in the vicinity of Maysville (Benton Co.) after rains on 14 March 2015. Only 2 sites were sampled (Figure 1), since all land in the area is privately owned.

At Site 1, several males were calling but only 1 female was seen on 14 March. At a nearby locale (Site 2), 2 amplexic pairs were observed on 15 March. On 16 March, 2 amplexic pairs and ~15 males were calling at Site 1 and males but no amplexic pairs were observed at Site 2. Numerous other breeding choruses were heard in the nearby area including across the Oklahoma border. The area around Maysville is a matrix of pastureland and agricultural land and seems to provide ample habitat to support a large population of crawfish frogs. At this area, the breeding season seems to occur in March. Bragg (1953) reported crawfish frogs breeding in adjacent northeastern Oklahoma during March and April.

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Figure 1. A) *Lithobates areolatus* breeding site near Maysville (Benton Co.), Arkansas in a cattle pasture (Site 1). B) *Lithobates areolatus* breeding site near Maysville (Benton Co.), Arkansas in a roadside ditch (Site 2). Note: these images were taken on 2 April 2015, ca. 2 weeks after the breeding choruses were observed, and the water level is lower than during the breeding event.

CLASS REPTILIA

Dactyloidae – Anoles

Anolis carolinensis (Voigt) – Northern Green Anole. On 3 June 2014, MBC discovered an adult male (SVL 63 mm) from South Arkansas Community College West Campus, 0.5 km S Center of El Dorado, Union Co., that had a bifurcated tail (Figure 2). Tail bifurcation, although rare, is known to occur in various lizards (see Trauth et al. 2014 and references therein). However, this is the first report of tail bifurcation of a lizard occurring in Arkansas.

Thamnophis proximus proximus (Say) – Western ribbon snake. An adult female (HSU Collection 1744, snout-vent length [SVL] = 535 mm) was captured 30 June 2014 from Long Lake at Woodville off AR St. Hwy 11, Lincoln Co. (34.123155°N, -91.690684°W). A total of 17 embryos was found and 13 removed from embryo sacs and measured: mean SVL = 132.5 ± 2.2

SD), range 130-137 mm. The remaining yolk masses were approximately 20 mm in diameter.

Trauth et al. (1994) reported a mean clutch size of 19.5 ($n = 17$, range 8-34 eggs) for Arkansas specimens, which was higher than reported in adjoining states. In Louisiana, Oklahoma and Texas, Fitch (1985) reported an average litter size of 11.6 ($n = 4$, range 4-24). In Texas, litters of 4-27 (mean 10-15) were reported (Werler and Dixon 2000). Our count from this individual snake is consistent with the higher clutch size reported for Arkansas specimens.



Figure 2. *Anolis carolinensis* exhibiting a bifurcated tail from Union Co., Arkansas.

Nerodia cyclopion cyclopion – Mississippi Green Water Snake. An adult female *N. c. cyclopion* (SVL = 750 mm) was captured by hand on 30 June 2014 from Cane Creek State Park off AR St. Hwy 11, Lincoln Co. (33.9166°N, -91.7657°W). A total of 13 embryos was found, and 3 were removed from the embryo sacs and each measured about 50 mm SVL. Intact embryo sacs ranged between 25-28 mm in diameter.

Based on only 3 specimens, Trauth et al. (1994) reported an average clutch size of 18.3 (range 8-34) for this uncommon snake in Arkansas. However, in the adjoining states of Louisiana, Oklahoma and Texas, Fitch (1985) reported a smaller average litter size of 11.6. Our count from this individual snake is consistent with the higher clutch size reported for Arkansas specimens.

Emydidae – Box and Freshwater Turtles

Terrapene triunguis (Agassiz) – Three-toed Box Turtle. Forms, excavated in soil or leaf litter, are used by box turtles to conceal themselves. One unusual form was discovered by MBC from vic. El Dorado, Union Co. that was used by at least 2 individual three-toed box turtles. On 4 September 2014, an adult male (carapace length [CL] 114.8 mm) was discovered occupying a form excavated in a metal hubcap filled

with leaf litter (Figure 3). On 12 September and again on 14 September 2014, another male (CL 102.2 mm) was found using the same form. Forms are not defended by resident turtles and multiple turtles may use the same form on sequential days (Dodd 2001). We suspect that this form was occupied by these turtles because the hubcap retained water and provided a moist environment in an otherwise more arid habitat.



Figure 3. Adult male Three-toed Box Turtle, *Terrapene triunguis* using an abandoned hubcap as a form.

CLASS AVES

Hirundinidae – Swallows and Martins

Petrochelidon pyrrhonota (Vieillot) – Cliff Swallow. The known breeding range of Cliff Swallows in Arkansas is extended northward in deltaic eastern Arkansas by 2 new county records (Connior et al. 2011). An expanded breeding range has been augmented by construction of concrete bridges that provide suitable attachment surfaces for mud nests (Tumblison 2007, 2009).

Jefferson Co.: On 3 July 2014, several dozen nests of Cliff Swallows were found under an overpass E of Pine Bluff, where US Hwy 425 and 63 intersect (GPS 34.20137°N, -91.96698°W).

Arkansas-Monroe counties: A check under the AR St. Hwy 1 bridge over the White River near St. Charles on 30 October 2014 revealed several nests of Cliff Swallows. These occurred on all-concrete construction at the tops of support pylons.

CLASS MAMMALIA

Soricidae – Shrews

Blarina brevicauda (Say) – Northern Short-tailed Shrew. A single adult female flea (*Doratopsylla*

blarinae) was collected from an adult *B. brevicauda* in Searcy Co. on 30 Aug. 2014. This appears to be the first report of an ectoparasite from *B. brevicauda* from Arkansas. However, this flea is a common ectoparasite of shrews and has been collected from the same host species in Missouri (Kollars et al. 1997) and from *B. carolinensis* from Arkansas (Connior et al. 2014a).

Blarina carolinensis (Bachman) – Southern Short-tailed Shrew. Three pregnant females collected 7-15 February 2013 in Union Co. had embryo counts of 2, 3, and 4. Connior et al (2014b) reported 3 embryos from a single female from this population. Embryo counts for this species range from 2-6 (McCay 2001).

Sciuridae – Squirrels

Tamias striatus (L.) – Eastern Chipmunk. One pregnant female collected from Marion Co. on 18 February 2013 contained 4 embryos. This coincides with spring breeding occurring in February, and reported average embryo counts of 4 to 5 (Snyder 1982).

Cricetidae – New World Mice

Peromyscus attwateri J.A. Allen – Texas Mouse. Little is known regarding reproduction of Texas mice in Arkansas, excepting Cockrum's (1952) report of lactating females in April and October from northwestern Arkansas. We collected 1 pregnant female from Searcy Co. that contained 4 embryos on 31 August 2014.

The flea *Orchopeas leucopus* was also collected from this population from 3 individuals on 17 January 2015. Although *O. leucopus* is a common flea of *Peromyscus* spp. and some other small mammals, this is the first report of this species from *P. attwateri*. In fact, only 1 species of ectoparasite, the laelapid mite *Androlaelaps fahrenheitsi* (reported under the synonym *Haemolaelaps glasgowi*), has been reported from *P. attwateri*, from Kansas (Long 1961) with no collections from Arkansas.

Peromyscus leucopus (Rafinesque) – White-footed Mouse. Three pregnant females with embryo counts of 3, 3, and 4 were collected vic. Mull, Marion Co. on 18 February 2013, which are typical litter sizes. We also collected the following ectoparasites from individuals in this population: an American dog tick (*Dermacentor variabilis*) larva and 2 fleas (*Orchopeas leucopus* and *Epitedia wenmanni*). Immature stages (larvae and nymphs) of *D. variabilis* are common parasites of small mammals in eastern North America (Kollars et al. 1997) and this tick recently was reported from Arkansas by McAllister et al. (2013) from *Sciurus*

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niger. Both fleas, *O. leucopus* and *E. wenmanni* and the tick, *D. variabilis*, have been reported previously from *P. leucopus* (Durden and Wilson 1991, Whitaker and Mumford 2009).

Ochrotomys nuttalli (Harlan) – Golden Mouse. Two pregnant females collected from Union Co. had embryo counts of 2 (7 February 2013) and 3 (5 February 2013), consistent with Linzey's (1968) mean litter size of 2.65. The tick, *D. variabilis*, the flea, *Ctenophthalmus pseudagyrtis*, and the laelapid mite, *Androlaelaps fahrenheiti* were collected from individuals in this population. These are common ectoparasites and have been reported previously from golden mice in other states, but these are the first ectoparasite records from this host in Arkansas (see Durden 2008 for review). Connior et al. (2014a) recently reported the flea *C. pseudagyrtis* from the shrew *Blarina carolinensis* and the eastern mole, *Scalopus aquaticus* in Arkansas.

Sigmodon hispidus Say and Ord – Hispid Cotton Rat. One adult male collected 9 February 2015 from Marion Co. was parasitized by immatures of the tick, *D. variabilis*, and adults of the flea, *C. pseudagyrtis*. Both of these species have been reported previously from *S. hispidus* (Clark and Durden 2002, Kollars et al. 1997) and from Arkansas.

Neotoma floridana (Ord) – Eastern Woodrat. One female collected in Polk Co. on 21 January 2012 was pregnant (embryo count was not recorded but the largest embryo was 19.8 mm in length). This provides evidence that woodrats reproduce during the winter at least in southern Arkansas. A single species of chigger mite, *Euschoengastia peromysci* was collected from this Polk Co. population, and a single species of laelapid mite, *A. fahrenheiti*, was collected from a woodrat from Union Co. on 8 February 2013. This chigger species has not been recorded previously from Arkansas but it has been recorded as an ectoparasite of *N. floridana* from some other states (Walters et al. 2011). *Androlaelaps fahrenheiti* is a common laelapid mite that parasitizes many species of North American mammals (Whitaker et al. 2007) and has been reported from eastern woodrats in other states previously (Durden et al. 1997).

Microtus pinetorum (Le Conte) – Woodland vole. One adult female captured in a pitfall trap in Izard Co. on 3 April 2008 had 2 young traveling with her; each weighed 4 g and were 45 mm in total length. These

young probably were only ~1-2 weeks old based on developmental and size descriptions of woodland voles (Smolen 1981).

Vespertilionidae – Vesper Bats

The following represent new county records of bats in Arkansas.

Corynorhinus rafinesquii (Lesson) – Rafinesque's Big-eared Bat. Conway Co.: a pregnant adult female captured by DBS on 22 May 2014 in a mist net set over a road in Sec. 4, T7N, R17W.

Faulkner Co.: 3 new records, including a male submitted for rabies testing from Conway on 2 September 1982, an adult male submitted for rabies testing from Greenbrier on 17 June 2002, and an adult male brought by a cat to a house in Mayflower and submitted for rabies testing on 25 July 2014.

Corynorhinus townsendii ingens Handley – Ozark Big-eared Bat. Boone Co.: 1 male bat observed by G. O'Hagan in a cave in Sec. 32, T20N, R18W on 14 May 1979. He observed another male in a cave in Sec. 10, T19N, R19W on 14 May 1979.

Franklin Co.: a single bat observed by WL Puckette in a crevice cave in Sec. 8, T11N, R27W on 11 June 1996, and a single bat he observed in another crevice cave in Sec. 12, T11N, R28W on 24 July 1996.

Eptesicus fuscus (Palisot de Beauvois) – Big Brown Bat. Arkansas Co.: an adult male submitted from St. Charles for rabies testing on 6 June 2013.

Grant Co.: new records are from individuals submitted for rabies testing. An adult male from 6 May 2002 and an adult female from 3 September 2002 originated from Sheridan. Also submitted from Grant Co. were an adult female collected 10 January 2012 and an adult female collected 10 April 2012.

Miller Co.: specimens submitted for rabies testing included an adult male submitted on 21 July 2005 and an adult male from Texarkana submitted on 28 March 2006.

Lasiurus noctivagans (Le Conte) – Silver-haired Bat. Boone Co.: an adult female submitted for rabies testing on 12 January 2012.

Monroe Co.: an adult female from Holly Grove submitted on 3 January 2008.

Lasiurus borealis (Müller) – Eastern Red Bat. Poinsett Co.: individuals submitted for rabies testing included an adult female and 3 juvenile females from Trumann sent 28 June 1991, and 1 juvenile female

submitted on 2 July 2008.

Lasiurus seminolus (Rhoads) – Seminole Bat. Saline Co.: 1 rabid adult male from Benton, submitted for rabies testing on 3 October 2002.

Scott Co.: during the Southeastern Bat Diversity Network Ouachita Bat Blitz, MK Clark captured 1 adult male in a mist net set over a pond on 1 August 2005 in Sec. 8, T2N, R28W.

Woodruff Co.: an adult female from Augusta submitted for rabies testing on 23 August 2002.

Myotis austroriparius (Rhoads) – Southeastern Myotis. Miller Co.: an adult female submitted for rabies testing on 28 June 2005.

Ouachita Co.: this bat was found by DAS and DBS in an old water well in Sec. 20, T11S, R18W, which sheltered this species on numerous occasions – 8 January 1999 (1 male), 17 December 1999 (1 male), 28 December 2009 (1 male and 2 females), and 22 March 2010 (2 males, 2 females, and 1 bat that escaped before its sex could be determined).

Pulaski Co.: an adult female was submitted for rabies testing on 1 August 2008.

Myotis grisescens A. H. Howell – Gray Bat. Cleburne Co.: an adult male submitted from Tumbling Shoals for rabies testing on 5 September 2013.

Franklin Co.: 2 of these bats were observed by WL Puckette in a crevice cave in Sec. 8, T11N, R27W on 3 January 1997, and he observed 2 in the same cave on 28 December 2004.

Marion Co.: 2 individuals were observed by G. O'Hagan in Blue Heaven Cave on 7 October 1978, and he captured 8 males in a mist net placed in the entrance of this cave on 13 June 1980. A maternity colony of an estimated 12,000 bats was observed by MJ Harvey in Summer Cave on 14 July 1982.

Izard Co.: 5 individuals were observed by DBS in a cave in Sec. 22, T16N, R8W on 7 March 2007.

Myotis septentrionalis (Trouessart) – Northern Long-eared Bat. Pulaski Co.: an adult female found alive hanging on an outside wall of the Arkansas Department of Health headquarters building in Little Rock was submitted for rabies testing on 4 September 2014.

Sevier Co.: an adult female brought to a cat owner's porch in Gillham, submitted for rabies testing on 24 July 2014.

Myotis sodalis Miller and G. M. Allen – Indiana Bat. Franklin Co.: on 2 January 1998, WL Puckette

observed 110 individuals of this Federally endangered species in a crevice cave in Sec. 8, T11N, R27W. He observed another individual in a nearby crevice cave in this section on this same date.

Marion Co.: on 10 November 1979, 5 of these bats were observed by G. O'Hagan in Elm Cave.

Sharp Co.: on 5 August 1980 a single male was observed by MJ Harvey in Morris Cave.

Perimyotis subflavus F. Cuvier – Tricolored Bat. This bat is now known from several new counties based on both submission for rabies testing and records gleaned from journals of mist-netting activities.

Carroll Co.: a juvenile female from Berryville was submitted for rabies testing on 10 September 1993, and another female was submitted on 18 September 2001. Also, a female from Green Forest submitted for rabies testing on 10 August 1999 was rabid.

Crawford Co.: a single Tricolored bat was observed by WL Puckette in a crevice cave in Sec. 11, T12N, R33W on 24 December 1996.

Faulkner Co.: an adult male was submitted for rabies testing on 30 September 1993, an adult female was submitted on 6 November 2002, another adult female on 17 September 2003, and a rabid adult female on 11 June 2004. All of these originated from Conway. Also, an adult female was submitted from Faulkner Co. on 31 May 2005.

Lafayette Co.: an adult male was captured by DAS inside a water well in Sec. 10 T17S, R23W on 2 January 2014.

Logan Co.: On 4 December 2004 DAS observed a male Tricolor Bat in a shelter cave, and on 22 January 2005 he found 3 in one crevice cave and 9 in another crevice cave on 12 March 2005, all on Magazine Mountain in Sec. 21, T6N, R25W (Saugey 2005).

Scott Co.: mist netting produced several records of this bat. On 1 August 2005, a juvenile female, an adult male, and a juvenile male were captured over a creek in Sec. 4, T2N, R30W, by L. Gatens as part of the Southeastern Bat Diversity Network Ouachita Bat Blitz. During the same bat blitz and on the same date, a juvenile female was captured by J. Szewcak in a mist net set over a creek in Sec. 12, T2N, R31W. An adult male was captured by DBS over a creek in Sec. 15, T4N, R27W on 2 August 2005. A juvenile male, an adult male, and a Tricolored Bat that escaped before its sex could be determined were captured by J. Szewcak during the bat blitz, over a creek in Sec. 28, T4N, R27W on 2 August 2005.

Van Buren Co.: 2 juvenile males were captured by DBS over the Little Red River in Sec. 26, T11N,

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R16W on 8 August 2005. An adult male was submitted for rabies testing on 15 April 2008.

Yell Co.: 1 adult male was captured by JL Jackson on 5 August 2003, during the Southeastern Bat Diversity Network Ouachita Bat Blitz, over a stream in Sec. 13, T2N, R25W. Also during the bat blitz, 1 adult female was captured over a stream in Sec. 14, T2N, R25W, by DA Miller on 6 August 2003.

Mollosidae – Free-tailed Bats

Tadarida brasiliensis (I. Geoffroy) – Brazilian Free-tail Bat. Cleburne Co.: an adult male was submitted for rabies testing on 12 August 2011.

Jefferson Co.: rabies testing of individuals submitted from a school in Pine Bluff on 10 April 2013 revealed 1 rabid adult male, 9 non-rabid adult females, and 11 non-rabid adult males.

Pope Co.: an adult female was submitted on 29 May 2009, an adult female was submitted on 7 February 2012, and an adult male from Russellville was submitted on 19 March 2014.

Union Co.: an adult female was submitted for rabies testing on 9 March 2009.

Mustelidae – Weasels and allies

Mustela frenata Lichtenstein – Long-tailed Weasel. Sealander and Heidt (1990) indicated that this species occurs statewide, but did not indicate any specimen records and commented that it is rare. A male specimen was captured in a chicken house near Rye, Bradley Co., in December 2013. Standard measurements were: total length, 420 mm; tail length, 140 mm; hind foot length, 43 mm; and ear length, 22 mm.

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Low-level Mercury Causes Inappropriate Activation in T and B Lymphocytes in the Absence of Antigen Stimulation

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Running Title: Low-level Mercury Induces Lymphocyte Activation

Abstract

The immune system primarily utilizes two cell types for adaptive immunity: T lymphocytes and B lymphocytes. T lymphocytes are activated when antigen presenting cells (APCs) present antigen to membrane-bound T cell receptors. B lymphocytes are activated when an antigen binds to receptors embedded in the plasma membrane. In both T and B cells this antigen binding crosslinks the receptor complexes and initiates the signal transduction cascade. These cascades frequently consist of a series of intracellular molecules becoming phosphorylated in a step-wise fashion. Once activated, these cells differentiate into effector cells that clear out the stimulating antigen. Mercury, which is a widespread environmental contaminant, is known to affect the immune system by increasing the potential for autoimmunity. The mechanisms triggered by low-level mercury exposure that cause an organism's immune system to attack its own tissues are currently unclear. In this study, populations of EL4 T cells and WEHI-231 B cells were exposed to multiple low-level concentrations of HgCl_2 for either 12 or 96 hours to mimic acute and chronic subtoxic exposure. Analytical intracellular flow cytometry was used to detect the presence of fluorescent-labeled antibodies bound to phosphorylated forms of multiple activation molecules in the signal transduction cascade. In the absence of antigenic stimulation, increased levels of activation molecules were present within both types of immune cells following low-level mercury exposure, indicating inappropriate activation of these cells by the mercury alone.

Introduction

Inorganic mercury (Hg) is a widespread environmental toxin. Methanogenic bacteria transform mercuric chloride into organic methylmercury (CH_3HgCl) and dimethylmercury ($\text{CH}_3\text{CH}_2\text{HgCl}$)

(Tchounwou et al. 2003). These organic compounds are prone to entering water supplies and food chains where humans are most likely to ingest them (Zahir et al. 2005). Mercury has well-documented neurotoxic and immunological effects on humans and mice (Rowley and Monestier 2005).

Immunological effects in rats and mice include elevated serum levels of immunoglobulin G1 (IgG1), immunoglobulin E (IgE) and the formation of renal IgG deposits (Hultman and Enestrom 1989). Autoantibody production, particularly antinucleolar autoantibodies, is characteristic of mercury-induced autoimmunity (Chen and von Mikecz 2000, Pollard et al. 1997). This autoimmunity is most often studied in mice that have H-2^s, H-2^b or H-2^d genetic backgrounds because they are predisposed to developing autoimmunity in response to heavy metal exposure (Hultman et al. 1994, Warfvinge et al. 1994, Abedi-Valugerdi and Moller 2000, Hansson and Abedi-Valugerdi 2004, Silva et al. 2005, Laiosa et al. 2007).

It has been shown in animal models that exposure to xenobiotics, including mercury, leads to the development of autoimmune diseases (Via et al. 2003, Silva et al. 2004). In humans, exacerbation of scleroderma, lupus and rheumatoid arthritis, as well as neurological dysfunction, has been documented (Verwilghen et al. 1992, Yokoo et al. 2003, Silbergeld et al. 2005, Copper et al. 2008). Mercury can also affect perinatal development when exposure occurs before birth (Dietert and Piepenbrink 2006). Our study examines longer exposure and subtoxic doses of murine lymphocyte cell lines to mercury, examining alterations in signal transduction molecules following exposure. This exposure level may be more like that which humans encounter in diet and environment.

Previous work in the available body of literature has focused on exposing immune cell lines to high concentrations of HgCl_2 for short durations of time. Several studies found attenuation in activation of WEHI-231 B cells and Jurkat T cells following *in vitro* exposure to mercury. Activation of lymphocytes

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commences with stimulation through their surface-bound antigen receptors, and can be artificially achieved via anti-Ig μ and anti-CD3 antibodies for B and T lymphocytes, respectively. Once the cells are activated, signal transduction cascades commence to push the cells into the cell cycle for proliferation. These signal transduction cascades frequently involve phosphorylation of cytoplasmic and/or cytoplasmic portions of membrane-bound proteins. In vivo, this proliferation would be accompanied by differentiation into effector forms (such as antibody-producing plasma cells and helper/cytotoxic T lymphocytes) to carry out the immune responses. (McCabe et al. 1999, McCabe et al. 2007, Ziemba et al. 2009, Limnander and Weiss 2011). In these studies, WEHI-231 B cells were exposed to 5 μ M HgCl $_2$ for 15 minutes and Jurkat T cells to 5 μ M HgCl $_2$ for 5 minutes. These studies indicated elevated activation in negative control samples when exposed to mercury alone in the absence of stimulation, but did not discuss these results (McCabe et al. 1999, Ziemba et al. 2009).

The mercury concentrations and exposure times of the McCabe and Ziemba studies are of a questionable physiologic relevance. It has been cited that 0.1 μ M mercury is a biologically relevant dose and that serum concentrations in exposed human populations are roughly 0.15 μ M mercury (Druet et al. 1989, Ratcliffe et al. 1996). In a later study by McCabe et al., exposure times were expanded somewhat to 30 minutes. Mercury levels used remained high at 20 μ M and 10 μ M (Gill et al. 2014). This 2014 study also utilized stimulation through the antigen receptor in addition to mercury exposure to examine signal transduction pathway alterations. The question our study attempts to answer is what effect longer exposure times with lesser amounts of mercury have on signal transduction pathway molecules in a B and T lymphocyte cell line. It is also important to note that our work examines the activation status of these molecules and cells in the absence of stimulation through the antigen receptor. We examined the effects of mercury exposure alone on the cells.

To expand upon the studies performed by McCabe et al., we investigated the activation of EL4 T lymphocytes (H-2^b) and WEHI-231 B lymphocytes (H-2^d) signal transduction molecules using mercury concentrations of 0.01, 0.1 and 1 μ M HgCl $_2$. Both lines are of an MHC haplotype susceptible to mercury stimulation of autoimmunity. The results of mercury on signaling molecules can be measured by the use of the phosphoflow method, wherein flow cytometric analysis of levels of phosphorylation of internal

signaling molecules is examined and quantified (Gill et al. 2014, Goldeck et al. 2013, Toapanta et al. 2012). To expand upon the exposure times used in the previously published studies, cells were incubated in mercuric chloride-laced media for either 12 (acute) or 96 (chronic) hours. Under these conditions, our findings indicate mercury alone causes significantly increased phosphorylation of several signal transduction molecules.

Materials and Methods

Cells

EL4 T cells or WEHI-231 B cells (obtained as a generous gift from Randy Hardy, Fox Chase Cancer Center) were cultured in RPMI-1640 media supplemented with 5% FBS, L-glutamine, Penicillin and Streptomycin, HEPES buffer and 2-mercaptoethanol. Cells were maintained in a 5% CO $_2$ incubator at 37°C. Trypan blue exclusion dye was used to ensure viability (~90%).

HgCl $_2$ Necrosis Experiments

EL4 and WEHI-231 cells were incubated in multiple concentrations (0 μ M, 0.01 μ M, 0.1 μ M, 1 μ M and 5 μ M) of HgCl $_2$ in the media for either 12 or 96 hours. Cells were harvested, pelleted at 1000 x g for 8 minutes and washed once in 1X Phosphate-Buffered Saline (PBS). Cells were resuspended in 10 μ g/mL propidium iodide and analyzed via flow cytometry to determine toxicity.

Antibodies

AlexaFluora488-labeled Zap-70(pY319)/Syk (pY352), PE-labeled ERK1/2(pT202/pY204), PE-labeled p38MapK (pT180/pY182) and PE-labeled PKC- α (pT638) were purchased from BD Bioscience laboratories.

Phosphospecific Flow Cytometry

Cells were incubated in HgCl $_2$ -laced media for either 12 or 96 hours. EL4 cells were exposed to 1 μ M HgCl $_2$ whereas WEHI-231 cells were exposed to 0.01 μ M, 0.1 μ M and 1 μ M HgCl $_2$. Density gradient centrifugation with Lympholyte-M ensured that only living cells were harvested and subjected to intracellular staining. Cells were fixed for 15 minutes with 1% paraformaldehyde, pelleted at 1000 x g and washed with PBS. Cell membranes were permeabilized using 1 ml ice-cold 100% v/v methanol for 10 minutes. Following permeabilization, 1 ml PBS was added to rehydrate the cells. Cells were pelleted at

1000 x g and washed an additional two times with PBS. Subsequently cells were exposed to 20 μ l antibody suspended in 30 μ l staining buffer (5% FBS in PBS) for 30 minutes on ice in total darkness, as per the manufacturer's protocol. Cells were washed once in PBS and resuspended in 1 ml staining buffer for analysis. The level of fluorescent antibody within the cells was detected using 488nm laser on an analytical flow cytometer (Partec GMmbH, Munster, Germany). FCS Express software (DeNovo Software, Los Angeles, CA, USA) was used to determine intensity of the fluorescence in each sample.

Statistics

All statistics were performed using JMP Statistical Analysis Software (S.A.S., Cary, NC, USA). For all EL4 data, means were compared using Student's t-tests assuming unequal variances. For all WEHI-231 data, means were compared using two-way ANOVA for block design. Tukey HSD *post hoc* analysis was performed following a significant finding. All data were expressed as means \pm 1 standard error and significance was taken as $p < 0.05$.

Results

Toxicity of HgCl₂ in EL4 and WEHI-231

Individual cell lines were incubated in HgCl₂-laced media at specified concentrations for either 12 or 96 hours. Cells were removed from HgCl₂-laced media and stained with propidium iodide to detect dead cells. Cell death studies indicated an increase in death numbers following exposure to HgCl₂, but it was shown that viable cells were still present within each sample (Table 1).

Table 1. Percent living cells following incubation in mercury-laced media for either 12 hours (acute) or 96 hours (chronic). Data are average of three replications with standard error indicated.

HgCl ₂ (μ M)	WEHI-231		EL4	
	Acute	Chronic	Acute	Chronic
0	98.6(+/-0.6)	97.8(+/-1.7)	93.4(+/-1.9)	89.5(+/-1.9)
0.01	70.7(+/-1.5)	84.2(+/-1.7)	29.7(+/-1.4)	21.2(+/-1.9)
0.1	61.6(+/-2.1)	81.7(+/-2.6)	15.8(+/-0.8)	13.1(+/-0.5)
1	40.5(+/-2.5)	67.3(+/-2.3)	9.38(+/-0.98)	9.3(+/-1.4)
5	32.2(+/-1.1)	40.5(+/-0.95)	4.92(+/-0.49)	5.7(+/-0.8)

Inappropriate Activation in EL4 T cells

EL4 T cells were incubated for either 12 or 96 hours in HgCl₂-free media and 1 μ M HgCl₂-laced

media. Analytical flow cytometry was used to detect antibodies bound to phosphorylated forms of signaling molecules ERK1/2, PKC α , and p38MAPK. In the acute treatments, all molecules had significantly higher mean signal strength when compared with control samples (Figure 1).

The preliminary studies of EL4 T cells included 4 treatment groups (0 μ M, 0.01 μ M, 0.1 μ M and 1 μ M HgCl₂). During these studies we found 0.01 μ M and 0.1 μ M HgCl₂ did not exhibit any deviation from the results of the negative control while 1 μ M HgCl₂ showed dramatic change (results for 0.01 μ M and 0.1 μ M not shown). For this reason, the lower concentrations were not tested further.

Chronic treatment with 1 μ M HgCl₂-laced media resulted in a significant increase in mean signal for pp38MAPK, but signal from 1 μ M HgCl₂-treated cellular pPKC α and pERK1/2 were not found to be significantly higher than control treatments (Figure 2). This finding is likely due to a high degree of variation between replicates.

Inappropriate Activation in WEHI-231 B cells

WEHI-231 B cells were incubated for either 12 or 96 hours in HgCl₂-free, 0.01 μ M, 0.1 μ M and 1 μ M HgCl₂-laced media. Analytical flow cytometry was used to detect antibodies bound to phosphorylated forms of signaling molecules SYK, ERK1/2, PKC α , and p38MAPK. SYK is an important tyrosine kinase in the B cell receptor signaling pathway upstream of ERK (Gill et al. 2014, Rosenspire and Stemmer 2010).

In the acute treatments, pSYK, pPKC α , and pp38MAPK were found to have significant differences in mean signal strength between treatments. For these three molecules, the negative control and 0.01 μ M HgCl₂ signals were not different from one another; however signal strength from 1 μ M HgCl₂ treatments were significantly elevated relative to the lower concentrations (Figure 3).

Although there was no significant difference in the treatments for pERK1/2, the trend of elevated signal strength in higher concentrations is present (Figure 3; Tukey HSD, levels not connected by same letter are significantly different).

In chronic treatments, all four signaling molecules exhibited significant differences in signaling strength within each group. In pSYK and pERK1/2, 0.1 μ M and 1 μ M HgCl₂ showed an increase in signal strength over the negative control and 0.01 μ M HgCl₂. The same elevated trend can be seen for pPKC α and pp38MAPK, however *post hoc* analysis did not find significant differences between treatments - this finding is likely

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due to a high degree of variation between replicates (Figure 4; Tukey HSD, levels not connected by the same letter are significantly different).

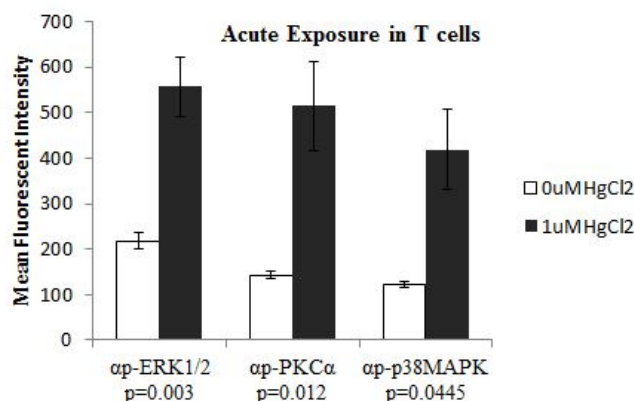


Figure 1. Fluorescent signal strength of three EL4 signal transduction molecules following 12 hour mercury exposure. Data shown are means \pm 1 SE; n = minimum 5. Statistically significant differences observed in p-ERK1/2, p-PKCα, and p-p38MAPK levels upon treatment with mercuric chloride, indicating an increase in signaling through mercury exposure alone.

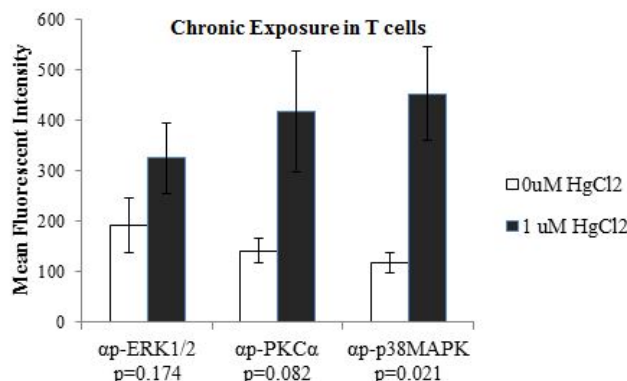


Figure 2. Fluorescent signal strength of three EL4 signal transduction molecules following 96 hour mercury exposure. Data shown are means \pm 1 SE; n=5. Statistically significant difference in p-p38MAPK levels only upon mercury exposure, indicating less of a long-term effect of mercury upon signaling molecule status.

Discussion

Antigen binding draws receptor molecules into close proximity and induces cross-linking that activates the cell. This assemblage of signaling molecules is described as the B cell signalosome (in B cells). It is not fully understood, but is generally accepted to occur upon activation through antigen receptors (Gill et al. 2014, Rosenspire and Stemmer 2010). Activated

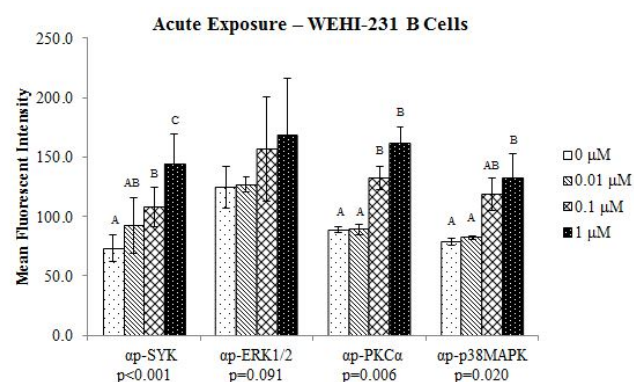


Figure 3. Fluorescent signal strength of four WEHI-231 signal transduction molecules following 12 hour mercury exposure. Data shown are means \pm 1 SE; n=3. Means not sharing the same notation are significantly different. No statistical difference was observed between treatment in pERK1/2 levels upon mercury exposure.

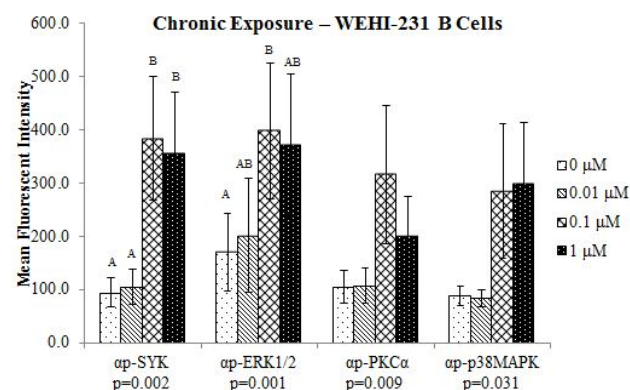


Figure 4. Fluorescent signal strength of four WEHI-231 signal transduction molecules following 96 hour mercury exposure. Data shown are means \pm 1 SE; n=4. Means not sharing the same notation are significantly different. Analysis showed an overall significant difference in phosphorylation for p-PKCα or p-p38MAPK, but no statistical difference between treatments was observed for p-PKCα or p-p38MAPK.

lymphocytes then differentiate into effector cells that act to clear out the antigen using a variety of mechanisms. This experiment shows that mercury is causing activation signal transduction events in the absence of antigen or artificial stimulation simulating an antigen. At this time, the mechanism by which this increased activation is occurring is unknown. It is suspected that mercury, which has an affinity for sulfhydryl groups, is binding to the sulfhydryl-rich receptors on the surface of lymphocytes (Mason et al. 1995). This may bring the receptors and their attached Src family kinases into close proximity, allowing

autophosphorylation to begin the signal transduction cascade (Figure 5).

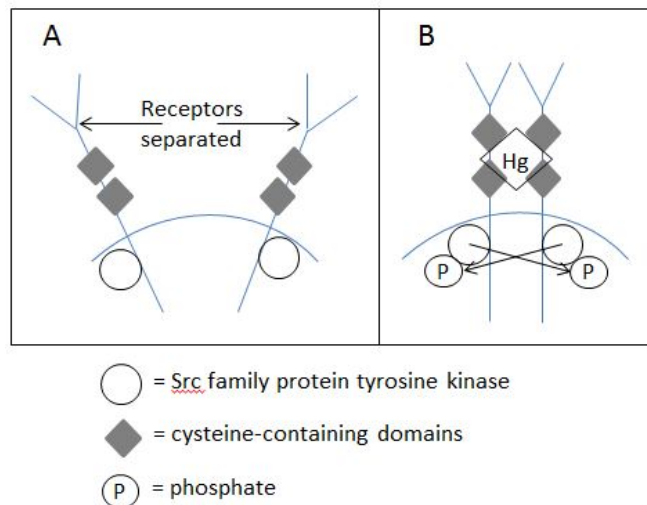


Figure 5. (A) Illustration of a lymphocyte in the absence of mercury. Surface receptors are separated and kinases are not in close enough proximity to autophosphorylate. (B) Illustration of a lymphocyte in the presence of mercury binding to sulfhydryl groups on cysteine amino acids in receptors. Binding may draw the receptors near and allow autophosphorylation events to occur between Src family kinases, including SYK.

It has been known for decades that mercury binds to sulfur atoms found in sulfhydryl groups on the amino acid cysteine (Boyer 1954). Until recently, the majority of mercury binding research focused on chemical interactions rather than the biological implications. Among several more recent works, one paper looked at how mercury binds to and alters the structure of human serum albumin (Li et al. 2007, Haase et al. 2015, Bal et al. 2013).

This brings about an interesting question related to the experiments performed here and elsewhere: If serum albumin can bind mercury, does the presence of the protein in tissue culture medium change the interpretation of the results presented here and in other studies? Tissue culture methods also frequently use 2-mercaptoethanol for support of cells, which exhibits capacity for binding mercury as well.

Answering this question is somewhat difficult. A study on zinc adsorption to serum proteins indicates that the ions can be largely soaked-up by the proteins in vitro. This phenomenon is characterized as a metal ion buffering capacity (Haase et al. 2015). Jurkat T cells, Raw 264.7, BV-2, and L929 cells were each examined for toxicity induction by zinc ion concentrations in increasing concentrations of fetal calf

serum and, separately, with differing amounts of bovine serum albumin. These studies indicated a decrease in cytotoxicity for each cell line exposed to zinc ions as culture protein levels were increased. Results also indicated a decrease in free ion concentration as protein levels increased in the medium. The authors cite several studies indicating that zinc ions are capable of inducing toxicity in cultured cells at nanomolar concentrations (Colvin et al. 2010, Schmidt et al. 2010, Bozym et al. 2010).

While this is an important finding, it may not be directly relevant to the results of our study. Mercury and zinc ions, while chemically related, are still different. They may be bound differentially by serum proteins, and the Haase study didn't extensively examine effects of mercury specifically. While several citations in the Haase study relate to other possible binding/depletion possibilities for zinc ions, these references don't address such concerns for mercury. A software package is available and was used in the Haase study for examination of zinc speciation, but mercury speciation and levels in different circumstances were not addressed to the same level of detail. It is unclear if lipids or phosphates would have an effect on media mercury levels as they do for zinc (Lu et al. 2012, Verweij 2012). It is also worth noting that only the Jurkat line is lymphocytic in nature. While it is lymphocytic, it is also a human (not murine) T cell line. Our studies, and those of McCabe, Gill, and Rosenspire, utilized murine lines, and reported more extensively on the B cell line results than the T cell line. The other three murine cell lines used in the zinc studies are macrophage, microglial, and fibroblast in cellular origin, respectively. Effects on one cell lineage may not be directly applicable to others.

Another study examined effects of mercury on WEHI 231 cells in the presence or absence of 2-mercaptoethanol (McCabe et al. 1999). The results of this study may also not be directly relevant to our particular methods for two reasons. The first is that this study utilized stimulation in vitro through the antigen-receptors on WEHI 231 cells via anti-Igμ antibodies. We did not use stimulation in our studies, relying instead on the effects of mercury alone. Second, while the concern about the presence of 2-mercaptoethanol and fetal bovine serum possibly acting as a metal ion buffer may be accurate, examination of several studies indicate neither is removed from the tissue culture methods used (Gill et al. 2014; McCabe et al. 1999; McCabe et al. 2007).

While we cannot answer definitively whether the presence of 2-mercaptoethanol and fetal bovine serum

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may act as a metal ion buffer in our studies, it may not matter. Previously published work has also used both compounds in similar experiments. Even more interesting is the idea at hand – if either compound sequesters mercury ions, effectively reducing their levels available for effects on the cell lines being cultured, it could mean that even lower doses of mercury actually reaching cells can alter signal transduction events in murine lymphocytes *in vitro*. The impact of that speculative idea on *in vivo* situations could be very intriguing indeed.

The most interesting finding in our study was that the acute treatment of the WEHI-231 B cells exhibited a dose-dependent response, in which increasing HgCl_2 concentrations resulted in more activation. While this result is probably to be expected, it has not been illustrated in work to this point. This dose-dependent response was not seen under chronic exposure – instead, there seems to be a threshold between $0.01\ \mu\text{M}$ and $0.1\ \mu\text{M}$ HgCl_2 in which signaling dramatically increases. This may be due to the fact that $0.01\ \mu\text{M}$ HgCl_2 is not a high enough concentration to affect WEHI-231 B cells, whereas $0.1\ \mu\text{M}$ HgCl_2 sufficiently draws B cell receptor complexes together to initiate the activation cascade. It could also be that the signaling molecules involved have become saturated and less responsive to continued exposure to mercury, requiring an even higher dose to initiate new signaling events. It is important to note that previous studies have not examined effects on signaling molecules following this length of *in vitro* mercury exposure. This represents a novel finding.

Most previous studies use a method for stimulating lymphocytes that simulated antigen/APC binding to artificially begin the signaling cascade (McCabe et al. 1999, Ziembra et al. 2009). In concurrence with these studies, we initially used anti-CD3 and anti-IgM antibodies to artificially stimulate EL4 and WEHI-231 cells, respectively. However, with increased HgCl_2 exposure time, it was not possible to differentiate between background stimulation due to HgCl_2 and stimulation due to the antibodies (results not shown).

Possible future research directions include moving into *in vivo* experiments using mice with an H-2^s background that would mimic a human immune system with a genetic predisposition to developing autoimmunity. Mice will metabolize HgCl_2 in a manner similar to humans which may result in different effects on immune cells than what is seen in this *in vitro* study. Closer examination of mRNA and/or protein levels for signaling molecules in acute

and chronic mercury exposure may also shed greater light on the effects observed.

Since it is not possible to prevent human exposure to environmental mercury compounds, it is necessary to gain insight into treatment and prevention of mercury-induced diseases. This study, and future work, will help us to characterize the effects of mercury at the molecular level and possibly find the mechanism(s) by which mercury causes diseases of the immune system.

Acknowledgements

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Foraging Behavior of Swainson's Thrushes (*Catharus ustulatus*) During Spring Migration Through Arkansas

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Running Title: Spring Foraging Behavior of Swainson's Thrushes in Arkansas

Abstract

Foraging behavior of Swainson's Thrushes on spring migration was studied in western Arkansas in the spring of 2013 and 2014. Observations were made in two forested field sites, one of them urban and the other suburban. The former had a significantly higher woody stem area (cm²) than the latter. For each foraging observation, the following three parameters were noted: Foraging Stratum (Ground, Shrub, Sapling, Sub canopy, and Canopy); Foraging Substrate (Ground/Litter, Herb, Foliage, Bark, and Air); and Foraging Maneuver (Glean, Probe, Dive/Glean, Hover, Jump Hover, and Hawking). We tested the hypotheses that these foraging variables differed significantly between the urban and suburban sites, and between the two years. These hypotheses were rejected for all three parameters. The consolidated data from both the sites and years revealed that a significantly higher proportion (67%) of the observations were on the Ground stratum, compared to the Shrub (13.7%) and Sapling strata (13%). Similarly, a significantly higher proportion (66%) of the foraging substrate used was Ground/Litter, followed by Foliage (16.7%) and Bark (15.8%). Gleaning was the most common foraging maneuver used (71.5%), and was significantly higher than Probing (12.3%) and Dive Gleaning (8.4%).

Introduction

Stopover areas are important links in the annual cycle of migrants since a migrant's survival and ultimate reproductive success hinges on suitability of habitat in these areas (Moore et al. 1990). The energetic costs of stopover for thrushes in north-bound spring migration in the United States have been shown to be higher than their flight costs due to cold weather and efforts involved in foraging (Wikelski et al. 2003). Habitat structure in stopover areas may influence foraging strategies adopted by the migrants, which in turn may

affect the rate at which they replenish their energy reserves. Urban parks and suburban woodlots serve as "convenience store" stopover sites, supporting migrants between short flights to higher-quality sites (Mehlman et al. 2005).

The Swainson's Thrush (*Catharus ustulatus*) breeds in the northern part of North America and migrates south in winter to Central and South America (Mack and Yong 2000). It is a common spring transient in Arkansas, observed in a variety of habitats, including yards in towns (James and Neal 1986). Although a common bird in much of North America, it has suffered widespread population declines (Mack and Yong 2000). Two factors may explain these declines: loss or fragmentation of breeding habitat (Wilcove 1988, Holmes and Sherry 1988), and also the possible anthropogenic landscape changes in its winter range in Central and South America (Robbins et al. 1989). Although several aspects of this species have been well-studied in its breeding grounds in North America (see review in Mack and Yong 2000), very little is known of its biology or habitat use while on migration. A thorough knowledge of the bird's ecology from its breeding, wintering, and migration range is needed to formulate sound management decisions pertaining to its conservation.

We studied the foraging behavior of Swainson's Thrushes during spring migration across western Arkansas in May 2013 and 2014. Our study had two objectives: 1) To quantify the foraging behavior of Swainson's Thrushes during their spring migration through Arkansas, and 2) to evaluate whether these foraging behaviors differ significantly between years and between sites with different vegetational structure.

Study Area and Methods

Foraging behavior was studied by modification of methods adopted by Holmes and Robinson (1988). Two forested sites were chosen for the study, an urban

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(Ruth Armstrong Nature Area) and a suburban (Fianna Hills) woodlot, both in Fort Smith (Sebastian Co.), Arkansas. The former is managed by underbrush and fallen wood removal, and is a 2.6-ha track of woodland in the middle of Fort Smith, at the junction of two busy roads (Rogers Avenue and Old Greenwood Road). The latter is a less busy strip of forested hillside in the south part of the city, abutting Glen Flora Way, approximately 1 km long and 50 m wide.

Foraging tactics of Swainson's Thrushes were observed between May 1 and 15 of 2013 and 2014, coinciding with their spring migration through Arkansas (James and Neal 1986). Observations were made early mornings (0700-0900 hrs) and late afternoons till twilight (1600-2030 hrs). For each foraging activity (defined as a bird observed to capture prey), three variables were noted: Foraging Stratum (Ground – 0m; Shrub – 0.1-2m; Sapling – 2.1-8m; Sub canopy 8.1-14m; and Canopy - >14m), Foraging Substrate (Ground/Litter, Herb, Foliage, Bark, Air), and Foraging Maneuver (Glean, Probe, Hover, Dive/Glean, Jump-hover, Hawk). We defined each maneuver as follows, after Holmes and Robinson (1988): "Glean" was defined as a standing or walking bird picking up food; "Probe", when the bird creates a disturbance in the substrate to get its food; "Hover", when the bird momentarily flutters in the air to get food from vegetation or bark; "Dive/Glean", when the bird swoops down from an elevated perch and picks up food from the ground; "Jump-hover", when the bird on ground jumps and picks up food, usually from underside of a leaf; and "Hawk", when the bird flies and catches an insect in mid-air. Each bird was followed for a maximum of 5 observations (as opposed to Holmes and Robinson's [1988] method of following birds as long as possible) to minimize the dependency bias associated with sequential observations (Morrison 1984). Data were not normally distributed and hence analyzed by nonparametric Kruskal-Wallis (Siegel and Castellan 1988) and Wilcoxon-Mann-Whitney (Mann and Whitney 1947) tests. We tested for any significant difference between sites for the same year, and between years for the same site.

Habitat structure of the urban and suburban site was quantified in May 2014 using the protocol described by James and Shugart (1970). We measured 4 habitat variables within circular 0.04 ha plots (11m radius) in both sites. The centers of the plots were chosen at random along a trail. Five plots were sampled in the smaller urban site and 10 plots in the suburban site. The variables measured were woody stem area (cm²), shrub understory density (/44m²), ground cover (%), and canopy cover (%). Woody stem area was measured by recording the diameter at breast height (DBH) of all stems >7.5 cm DBH within the plot by using a DBH tape. Shrub density was determined by counting the number of stems at breast height (1.2 m) along two 1-m wide orthogonal transects through the center of the plot, the direction of the transects chosen by the random twist of a compass dial; ground and canopy cover was quantified by taking 40 presence or absence readings of green vegetation in the two aforementioned transects sighted through an ocular tube at random points in each plot. Differences between urban and suburban plots were tested for significance using Wilcoxon-Mann-Whitney test (Mann and Whitney 1947).

Results

Vegetation structure.

The woody stem area (cm²) was significantly higher ($P = .0006$) in the urban plot than in the suburban plot (Table 1). However, canopy cover, ground cover, and stem density were not significantly different between the sites ($P > 0.08$).

Foraging behavior.

We collected 226 foraging sequences of 1 to 5 observations as follows: 66 (29.2%) of the sequences yielded only one observation, 49 (21.7%) yielded 2, 31 (13.7%) yielded 3, 16 (7%) yielded 4, and 64 (28.3%) yielded the self-imposed maximum limit of 5 observations. The three foraging variables were not significantly different between the two years, and between the two sites ($P > 0.6$; Kruskal-Wallis Test; Figs.1-3).

Table 1. Analysis of 4 vegetational characteristics in the urban and suburban study sites. Underlined value represents significant difference between the two sites.

Vegetational characteristic	Urban plot (mean ± SE)	Suburban plot (mean ± SE)	<i>P</i> *
Woody stem area (cm ²)	263,822 ± 27,120	77,634 ± 14,839	<u>0.0006</u>
Stem density (/44m ²)	13.4 ± 5.9	24.4 ± 7.4	0.337
Canopy cover (%)	88 ± 3.6	78.5 ± 3.1	0.089
Ground cover (%)	49 ± 6.5	37.7 ± 6.5	0.127

*Wilcoxon-Mann-Whitney test

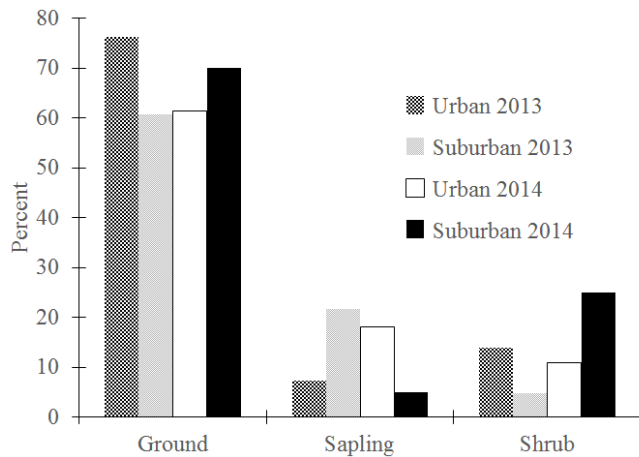


Fig. 1. Swainson's Thrush use (%) of main forest strata for foraging in 2013 and 2014 in the urban and suburban sites. Other strata were rarely used (see Table 2) and not included here.

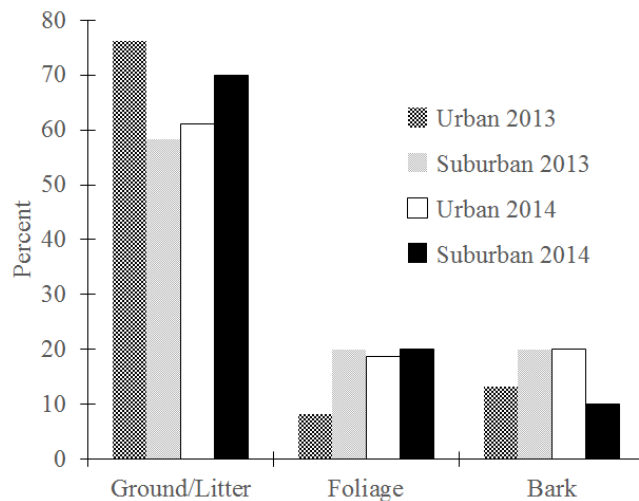


Fig. 2. Swainson's Thrush use (%) of three most common substrates for foraging in 2013 and 2014 in the urban and suburban sites. Other substrates were rarely used (see Table 3) and not included here.

Because the differences were not significant, we combined the data from both the years and from both the sites (Tables 2-4, $n = 637$ observations). Since the first observation in any foraging sequence tends to be biased toward conspicuous maneuvers like flying (Holmes and Robinson 1988), we tested for any significant difference between the 1st and the subsequent (2nd through 5th) observations (Tables 2-4) for all three foraging variables. The differences were not significant ($P > 0.83$, Wilcoxon-Mann-Whitney test, $W = 12, 14$, and 18 for stratum, substrate and maneuver, respectively). Hence, we combined the 1st observation data ($n = 226$) with the subsequent observations ($n = 411$) for all the analyses and

interpretations (based therefore on a total of 637 observations; Tables 2-4).

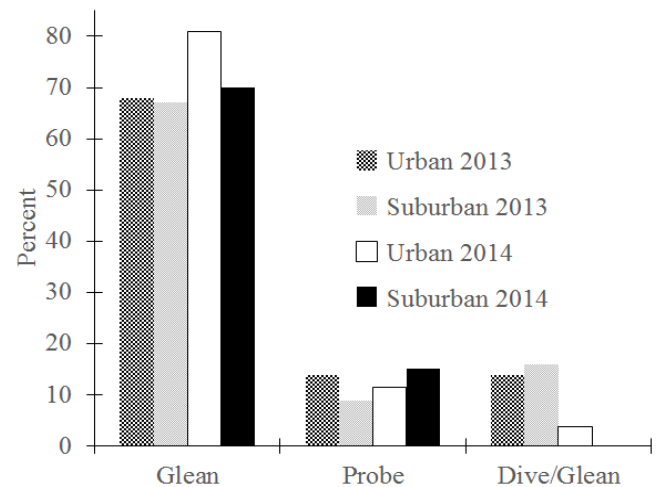


Fig. 3. Three most common foraging maneuvers used (%) by Swainson's Thrush in 2013 and 2014 in the urban and suburban sites. Other maneuvers were rarely used (see Table 4) and not included here.

A significantly higher proportion (67%) of the pooled observations were on the Ground stratum, compared to the Shrub (13.7%) and Sapling (13%) strata ($n = 637$ observations, Table 2). Similarly, a significantly higher proportion (66%) of the foraging substrate used was Ground/Litter, followed by Foliage (16.7%) and Bark (15.8%) ($n = 637$ observations, Table 3). Gleaning was the most common foraging maneuver used (71.5%), and was significantly higher than Probing (12.3%) and Dive Gleaning (8.4%) ($n = 637$ observations, Table 4).

Discussion

The Swainson's Thrush has generally been considered a "ground" (Holmes et al. 1979) or "near-ground" (Mack and Yong 2000) forager, and this is supported by our data, with 67% of observations on the ground. However, it is noteworthy that more than a quarter (26.7%) of our observations were 0.1-8m above ground, with some (6.2%) observations extending even higher, into the sub canopy and canopy. We occasionally found the birds in mixed hunting parties far above the ground, hopping along boughs to pick prey off bark, or hopping along horizontally and then flying to vertical boles to glean prey off bark or adjoining foliage. Ten per cent of all prey attacks were in the forest canopy in Holmes and Robinson's (1988) study (hereafter referred as Hubbard Brook study or

Spring Foraging Behavior of Swainson's Thrushes in ArkansasTable 2. Vertical distribution (% prey attacks in each stratum) of Swainson's Thrush foraging: data shown as 1st, subsequent (2nd through 5th), and ALL (combined) prey attacks on observed foraging sequences.

Stratum	1 st (n = 226 observations)	Subsequent (n = 411 observations)	All (n = 637 observations)
Ground	57.5	68.6	67
Sapling	22.6	12.2	13
Shrub	11.1	11.2	13.7
Sub canopy	6.2	4.6	4.4
Canopy	2.7	3.4	1.8

Table 3. Foraging substrate (% prey attacks in each substrate) used by the Swainson's Thrush: data shown as 1st, subsequent (2nd through 5th), and ALL (combined) prey attacks on observed foraging sequences.

Substrate	1 st (n = 226 observations)	Subsequent (n = 411 observations)	All (n = 637 observations)
Ground/litter	57.5	67.6	66
Foliage	18.1	16.3	16.7
Bark	23.5	15.1	15.8
Herb	0	0.5	0.3
Air	0.9	0.5	0.8

Table 4. Prey-attack maneuver (% prey attacks by maneuver type) of the Swainson's Thrush: data shown as 1st, subsequent (2nd through 5th), and ALL (combined) prey attacks on observed foraging sequences.

Maneuver	1 st (n = 226 observations)	Subsequent (n = 411 observations)	All (n = 637 observations)
Glean	74.8	75.2	71.5
Dive/Glean	12.4	15.1	8.4
Probe	5.3	5.4	12.3
Hover	4.4	2.9	4.7
Jump-Hover	2.2	1.0	2.3
Hawk	0.9	0.5	0.8

site), a much higher proportion than ours (1.8%) ostensibly because of the more vertical complexity of vegetation in that more mature New Hampshire site. This species is more arboreal than other *Catharus* thrushes (Mack and Yong 2000), and our data compared with other studies suggest that the bird increases its arboreal activity in forests with more vertical heterogeneity (foliage height diversity). The fact that the birds in the Hubbard Brook study foraged on the ground less (<50%) and attacked prey more often on tree foliage than our study (32 vs. 16.7%, respectively) may also be a reflection of more foraging opportunities available in the higher strata of more complex vertical environments.

The frequency of gleaning was strikingly different between our results (71.5%) and from that of the Hubbard Brook study (25%). This could be because both our sites had straight trails that gave us

unimpeded views across a wide area, allowing us to see ground-foraging Swainson's Thrushes even far away. That may not have been the case in the more mature Hubbard Brook site. The preponderance of foliage cover in the Hubbard Brook site may also account for the high proportion of hovering (37.9%) in that study compared to ours (4.7%).

Holmes and Robinson (1988) argued that longer sequence data are necessary to record rare foraging maneuvers like jump-hover or hawking. However, longer sequences are likely to skew results with unusually common observations and pseudoreplication. There were occasions when we could have obtained dozens (if not more) of observations from small flocks of Swainson's Thrushes gorging on ripe Mulberry (*Morus* spp.) fruits from one particular tree in the suburban site for both years. This would have skewed the results to reflect an unusually high proportion of

foliage gleaned (albeit of fruits and not insects) at strata higher than the shrub level. Besides, as indicated earlier, longer sequence data in general can be biased because the observations tend to be dependent on one another (Morrison 1984). Nevertheless, it is possible that behaviors recorded very rarely in our study, like hawking (0.8%) and hovering (4.7%) were less frequent than in the Hubbard Brook study (4.5% and 37.9%, respectively) partly because we did not follow the birds for more than 5 observations. The much more complex and mature nature of the Hubbard Brook site may also be responsible for the discrepancy, as discussed earlier.

Although four observers (RK, SW, and two field assistants) carried out the observations, RK did 72% of the observations in 2013, and SW did 86% in 2014. Since there was no significant difference between the years, we can state with confidence that any bias due to inter-observer variability was minimal.

Yong and Moore (1990) reported “foot-quivering” as a foraging maneuver in *Catharus* thrushes including Swainson’s during migratory stopover in Louisiana, and suggested that it functions in flushing prey. We did not observe this behavior, nor was it observed in the Hubbard Brook study. It is possible that this behavior is exhibited only after a long migratory flight (like trans-Gulf) when energy reserves are severely depleted, and when any extra energy obtained by such behaviors is invaluable and may make a difference in survival.

Mack and Yong (2000) reported that the Swainson’s Thrush inhabits a wider variety of habitats in migration than during the breeding season. We have seen the birds foraging in shrub-free grassy meadows with a scattering of mature planted oak *Quercus spp.* and other trees at the University of Arkansas - Fort Smith campus and in Tillis Park in the city. James and Neal (1986) observed the birds foraging in lawns, cemeteries, and urban woodlots in Arkansas. This wide habitat use during spring migration seems in contrast to the relatively more restricted usage in their breeding (mainly coniferous forests) and wintering (mainly primary rain- or semideciduous forest) grounds (Unitt 1984, Ramos and Warner 1980, Howell and Webb 1995). It also suggests that dense undergrowth may be an important but not a “main determinant” (Mack and Yong 2000) of habitat during migration.

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The Eastern Boxelder Bug, *Boisea trivittata* (Hemiptera: Rhopalidae): Confirmation in Arkansas

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Running Title: The Eastern Boxelder Bug in Arkansas

The documented hemipteran fauna of Arkansas has grown tremendously since taxa listed in Henry and Froeschner (1988). Several more recent reports have documented new records for the state (see Chordas et al. 2014). Although common and widespread, Chordas et al. (2005) pointed out that the eastern boxelder bug, *Boisea trivittata* (Say, 1825) was not included in the Arkansas list by Henry and Froeschner (1988). Although surprising, given the commonality of this bug, our literature search did not contradict the notion of the lack of a refereed published report of this species for Arkansas. Here, we document *B. trivittata*, with deposited voucher specimens, for the state.

On 19 March 2014, CTM collected numerous plant bugs from a terrestrial habitat at Flint Creek, SW of Gentry off county road 10, in Benton County (36°14'23.23"N, 94°30'00.03"W, elevation 330 m). Bugs were collected with a standard insect sweep net, placed in 70% ethanol and forwarded to SWC for identification. Voucher specimens were deposited into the C.A. Triplehorn Insect Collection (The Ohio State University, Columbus, Ohio). Brimley (1938), Henry and Froeschner (1988), Maw et al. (2000), Grimnes et al. (2003), Palazzo and Setzer (2009) and a WorldCat literature search were used for published distribution references.

Bugs were identified as males, females and nymphs of *B. trivittata*. Numerous specimens were collected along a cobble and sandy habitat from near the shore of Flint Creek (Fig. 1). We estimate an aggregation of over 500 bugs were in and among this habitat.

Boisea trivittata is a scentless plant bug that feeds mainly on the seeds of maple (*Acer* spp.) and soapberry (*Sapindus saponaria*) trees and are occasionally an urban nuisance pest around homes. An on-line image search for "*Boisea trivittata*" will yield multiple and very nice color photos of the species; thus, we do not include an image here.

In North America, *B. trivittata* is native to most of

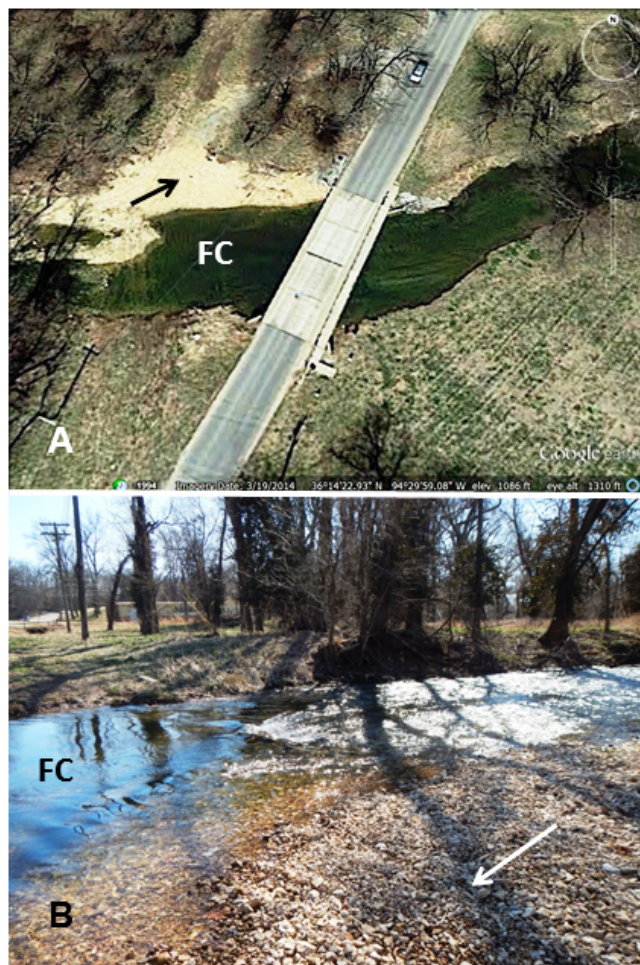


Figure 1. Collection site at Flint Creek (FC) where a large aggregate of *Boisea trivittata* was encountered. A. GoogleEarth aerial view of shoreline (arrow). B. Ground view of site looking southwest showing cobble and sand on shoreline (arrow).

the continental United States and Canada, excluding the very western parts (Fig. 2). Although a photograph and note about this species is posted on the University of Arkansas Arthropod Museum website (see <http://www.uark.edu/ua/arthemuse/boxbug.html>), insofar as we were able to determine, we provide the first literature record for *B. trivittata* in Arkansas.

The Eastern Boxelder Bug in Arkansas

A tabulation of literature geographic distribution records for *B. trivittata* (Fig. 2) includes 39 records, 35 in the USA and four records in Canada as follows: Alabama, Arizona, **Arkansas (Confirmation Record)**, Colorado, Connecticut, Florida, Illinois, Indiana, Iowa, Kansas, Minnesota, Nebraska, New Hampshire, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oklahoma, Maryland, Massachusetts, Michigan, Mississippi, Missouri, Montana, Pennsylvania, Rhode Island, South Dakota, Tennessee, Texas, Utah, Virginia, West Virginia, Wisconsin. *Canada*: Alberta, Manitoba, Ontario, Saskatchewan.

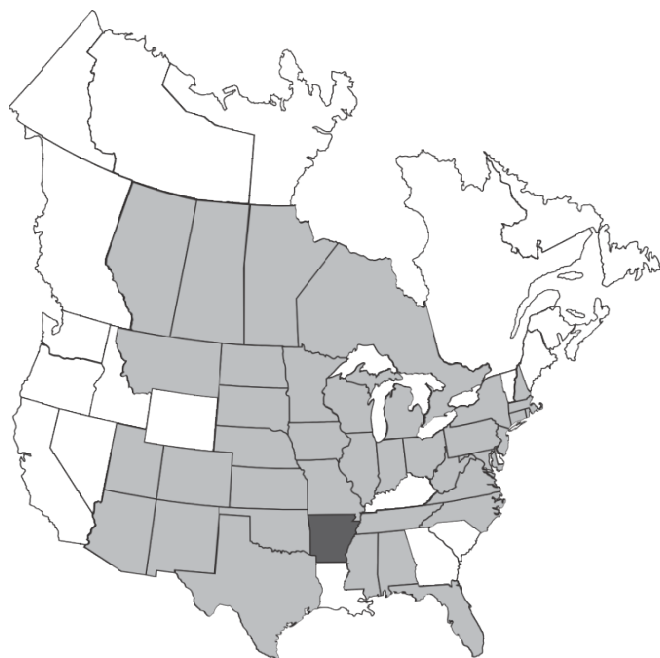


Figure 2. Distribution map of *Boisea trivittata* north of Mexico. Light shading (previous records); dark shading (confirmation record).

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Acanthocephalan Parasites of Select Fishes (Catostomidae, Centrarchidae, Cyprinidae, Ictaluridae), from the Arkansas and White River Drainages, Arkansas

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Running Title: Acanthocephala of Arkansas Fishes

Although there are reports of acanthocephalans from Arkansas game fishes (Becker et al. 1966, Becker and Houghton 1969, Bone 1974, Becker and Cloutman 1974, Cloutman 1975) little is known about the acanthocephalan (thorny-headed worm) parasites of non-game fishes of Arkansas. McAllister et al. (2008, 2014a) reported acanthocephalans from the Pirate Perch, *Aphredoderus sayanus* from the Caddo River, Clark County, and Rolling Fork River, Sevier County, respectively. In addition, McAllister et al. (2014a) reported unknown cystacanths in Blackspot Shiner, *Notropis atrocaudalis* and Yellow Bullhead, *Ameiurus natalis* from Nix Creek at Texarkana, Miller County. McAllister et al. (2014b) reported *Acanthocephalus tahlequahensis* in the Banded Sculpin, *Cottus carolinae* from Flint Creek, Benton County. More recently, McAllister et al. (2016) found unknown cystacanths in Northern Studfish (*Fundulus catenatus*) from Crooked Creek, Marion County. We are not aware of additional reports of acanthocephalans from non-game Arkansas fishes (see Hoffman 1999). Here, we report five new host records as well as two new distributional records for acanthocephalans from non-game fishes of the state.

Between March and June 2014, the following 20 fishes were collected from the Arkansas (Benton County) and White River (Independence County) drainages and examined for helminth parasites (sample sizes in parentheses): **CATOSTOMIDAE**: Highfin Carpsucker, *Carpionodes velifer* (2), Black Redhorse, *Moxostoma duquesnei* (1); **CENTRARCHIDAE**: Shadow Bass, *Ambloplites ariommus* (1); **CYPRINIDAE**: Central Stoneroller, *Camptostoma anomalum* (11); **ICTALURIDAE**: Slender Madtom, *Noturus exilis* (5). Fishes were collected with backpack electrofishers, dipnets or 6.1 m seine (3.2 cm mesh).

They were placed in habitat water and necropsied within 24 h. We followed accepted guidelines for the use of fish in research (AFS 2004) and specimens were overdosed with a concentrated Chloretone solution, measured for total length (TL) and a mid-ventral incision from anus to gill slit was made to expose the gastrointestinal tract and other internal viscera which was removed, placed in a Petri dish containing 0.6% w/v saline and examined with a stereomicroscope. Acanthocephalans were placed in dishes containing distilled water overnight, transferred to 70% v/v ethanol and sent to MAB for identification. They were stained with acetocarmine and mounted in Canada balsam. Voucher specimens were deposited in the Harold W. Manter Laboratory (HWML) of Parasitology, University of Nebraska-Lincoln. Host voucher specimens were preserved in 10% v/v formalin, transferred to 40% v/v ethanol, and deposited in the fish collection of Henderson State University Museum (HSU), Arkadelphia. Prevalence, mean intensity, and range of infection are provided and are in accordance with terminology given in Bush et al. (1997).

We collected four species of acanthocephalans from the intestinal tract of five species of fishes. The following is an annotated list of data as follows: host and TL (mean \pm 1SD range, when available), prevalence, intensity (mean \pm 1SD range, when available), collection site, collection date, HWML accession number.

Acanthocephala: **Palaeacanthocephala:**
Echinorhynchidae: *Acanthocephalus tahlequahensis*
Oetinger and Buckner, 1976

Ambloplites ariommus 35 mm TL, 1/1 (100%), 1 worm, collected on 20 Mar., 2014 from Flint Creek off

Acanthocephala of Arkansas Fishes

Fairmont Road at Springtown, Benton County (36°15'9.9"N, 94°26'25.8"W), HWML 75382.

Noturus exilis 75 mm TL, 1/5 (20%), 1 worm, collected on 20 Mar., 2014 from Flint Creek S of Gentry off US 59, Benton County (36°14'33.8"N, 94°29'14.9"W), HWML 75383.

The type host of *A. tahlequahensis* is the Sunburst Darter, *Etheostoma mihileze* (formerly *E. punctulatum*) from Black Fox Creek, Cherokee County, Oklahoma (Oetinger and Buckner 1976). Other reported hosts include the Redspot Chub (*Nocomis asper*), Cardinal Shiner (*Luxilus cardinalis*, formerly *Notropis pilsbryi*), and Orangethroat Darter (*Etheostoma spectabile*) (Oetinger and Buckner 1976). As noted previously, McAllister (2014b) reported *A. tahlequahensis* from *C. carolinae* from Flint Creek. However, McAllister et al. (2015) did not report *A. tahlequahensis* from 43 *N. exilis* collected between 2012–2013 from Benton, Marion and Searcy counties. Obviously, there is little host specificity as this acanthocephalan has now been reported from fishes in the families Centrarchidae, Cottidae, Cyprinidae, Ictaluridae and Percidae. Thus, we document two new host records for *A. tahlequahensis*.

Eoacanthocephala: Neoechinorhynchida:
Neoechinorhynchidae: *Neoechinorhynchus* sp.

Moxostoma duquesnei 382 mm TL, 1/1 (100%), 1 male worm, collected 5 Apr., 2014 from below Lock and Dam #1, White River at Batesville, Independence County (35°45'20.8"N, 91°38'17.3"W), HWML 75375.

Unfortunately, without females, we are unable to identify this worm beyond genus. However, this is the first time any acanthocephalan has been reported from *M. duquesnei*; therefore, we document a new host record.

***Neoechinorhynchus prolixus* Van Cleave and Timmons, 1952**

Carpionodes velifer 280 mm TL, 1/2 (50%), 4 female worms, collected 5 Apr., 2014 from below Lock and Dam #1, White River at Batesville, Independence County (35°45'20.8"N, 91°38'17.3"W), HWML 75374.

This acanthocephalan was described by Van Cleave and Timmons (1952) from River Carpsucker, *Carpionodes carpio* from Oklahoma. It has also been reported from Shorthead Redhorse, *Moxostoma macrolepidotum*, Fathead Minnow, *Pimephales promelas*, Quillback, *Carpionodes cyprinus* and *C. carpio* from Iowa, Nebraska, North Dakota and Oklahoma (Self and Timmons 1955, Kritsky et al. 1972, Barnhart et al. 1976, Samuel et al. 1976, Holloway and

Hagstrom 1981, Nickol and Samuel 1983, Forstie and Holloway 1984). We report a new host record as well as a new distributional record for *N. prolixus*.

***Paulisentis* sp.**

Campostoma anomalum 152.0 ± 6.3, 145–160 mm TL, 4/11 (36%), 5.8 ± 6.2, 4 males, 7 females, collected on 13 Jun., 2014 from Flint Creek off Fairmont Road at Springtown, Benton County (36°15'9.9"N, 94°26'25.8"W), HWML 75373.

Our specimens exhibit morphological characteristics shared by *Paulisentis fractus* Van Cleave and Bangham and *Paulisentis missouriensis* Keppner (Van Cleave and Bangham 1949, Keppner 1974). Unfortunately, without additional specimens, we cannot determine what species of *Paulisentis* is present at this time. However, we document a new host and distributional record for *Paulisentis* sp.

In conclusion, we document new information on acanthocephalan parasites of non-game Arkansas fishes while also adding to information on their helminths, which we know little about. With additional surveys, we expect to increase the acanthocephalan fauna of the state as well as possibly providing description of new species.

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Helminth Parasites of the Blackspotted Topminnow, *Fundulus olivaceus* (Cyprinodontiformes: Fundulidae), from the Interior Highlands of Arkansas

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Running Title: Helminths of *Fundulus olivaceus*

The Blackspotted Topminnow, *Fundulus olivaceus* (Storer) is a medium-sized fish that ranges in the Mississippi River basin from eastern Tennessee, western Kentucky, southern Illinois, and central Missouri south through eastern Oklahoma and Texas, Arkansas, Mississippi, and Alabama to the Gulf of Mexico (Page and Burr 2011). In Arkansas it is one of the most common and widespread fishes, occurring in all river drainages with a statewide distribution (Robison and Buchanan 1988). This fish prefers lentic habitats, is typically found in lower order streams, prefers areas near the land/stream margin, and is a surface feeder on insects and small crustaceans (Rice 1942).

Although information is available on the ecology of *F. olivaceus* (see Etnier and Starnes 1993, Pflieger 1997), little is known about its helminth parasites. As far as we can determine, only a single monogene, *Gyrodactylus megacanthus* Wellborn and Rogers, from Alabama, Arkansas and Mississippi and an acanthocephalan, *Neoechinorhynchus cylindratus* (Van Cleave) Van Cleave, have been reported from this topminnow (Wellborn and Rogers 1967, Hoffman 1999). Herein, we report four new host records as well as two new geographic locality records for parasites collected from *F. olivaceus*.

Between May 2014 and June 2015, 44 juvenile and adult *F. olivaceus* (mean \pm 1SD, range = 56.2 \pm 14.0, 31–83 mm total length [TL]) were collected using a backpack electroshocker, a dipnet, or 6.1 m seine (3.2 cm mesh), from the Ouachita River drainage of Middle Fork of Saline River and Bear, Mill and Walnut creeks, Garland County (n = 38), the Caddo River, Clark County (n = 4), and the White River drainage of

Crooked Creek, Boone County (n = 2). Specimens were placed in containers with cool aerated water from their collection site and necropsied within 24 hr. We followed accepted guidelines for the use of fish in research (AFS 2004); specimens were overdosed by immersion in a concentrated chlorotone solution and a mid-ventral incision was made to expose the gastrointestinal tract and internal viscera. Monogenes were removed with minuten nadeln from the gills of select fish (n = 17) preserved in 10% formalin from Bear Creek, and mounted in Gray and Wess medium stained with Gomori's trichrome. Digeneans from the intestine were fixed in near boiling tap water without coverslip pressure, transferred to 70-95% v/v ethanol, stained with acetocarmine and mounted in Canada balsam. Nematodes were placed on a glass slide in a drop of undiluted glycerol for identification. Voucher specimens of parasites were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska. Host voucher specimens were deposited in the Henderson State University (HSU) fish collection, Arkadelphia, Arkansas. Prevalence, mean intensity \pm 1SD, and range of infection are provided and are in accordance with terminology given in Bush et al. (1997).

Thirty-one of 44 (70%) of the *F. olivaceus* harbored one of three helminths, including 29 (66%) with the digenean, *Creptotremas* sp. in the intestines, one (2%) with the nematode, *Eustrongylides* sp. in the mesentery, and one (2%) with the nematode, *Rhabdochona cascadiella* in the intestines. In addition, 2 of 17 (12%) from one site in the Ouachitas harbored the monogene, *Salsuginus umbraensis*. Of the sites collected, only Bear and Crooked creeks provided

positive hosts. The following is an annotated list of data as follows: host total length (TL, mean \pm 1SD, range), prevalence, intensity (mean \pm 1SD), collection site, collection date(s), HWML accession number.

Monogeneoidea: Dactylogyrida: Ancyrocephalidae: *Salsuginus umbraensis* (Mizelle, 1938) Murith and Beverley-Burton, 1985 (Fig. 1)

65.0 \pm 4.2, 62–68 mm TL, 2/17 (12%), 1.0 \pm 0.0, range 1, Bear Creek at Bear, Garland Co. (34.535034°N, 93.286517°W). 30 May 2014, HWML 75372.

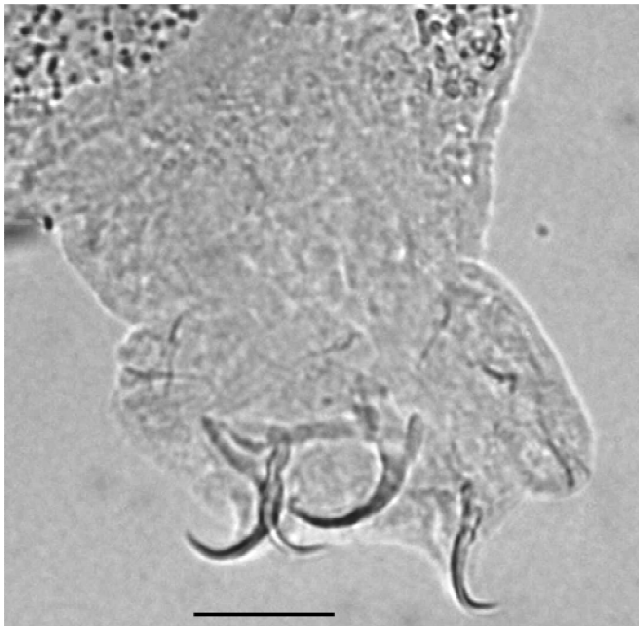


Figure 1. *Salsuginus umbraensis* from *Fundulus olivaceus*. Note hamuli. Scale bar = 20 μ m.

Based on the size and shape of the hamuli (Fig. 1) (dorsal and ventral hamuli 18–19 and 19–21 μ m long, respectively; angle of superficial/deep root notch about 60°; deep root forming a distinct thumb-like projection; blades long and thin, smoothly curved, thinner in dorsal hamulus), our specimens closely resemble the description of *S. umbraensis* provided by Mizelle (1938) and Murith and Beverley-Burton (1985) from Blackstripe Topminnow, *Fundulus notatus*. In addition, *S. umbraensis* has been reported from *F. notatus* from Illinois (Mizelle 1938, Murith and Beverley-Burton 1985), Louisiana (Duobinis-Gray and Corkum 1985) and Kentucky (Kozel and Whitaker 1985). This is the first report of *S. umbraensis* from *F. olivaceus* and Arkansas.

In spite of the high host specificity of species of *Salsuginus* (Murith and Beverley-Burton 1985), the very close phylogenetic relationship of *F. notatus* and

F. olivaceus (Cashner et al. 1992) may enhance the ability of *S. umbraensis* to parasitize both species of hosts. Alternatively, the two specimens observed in this study may represent a cryptic undescribed species warranting further study with additional specimens.

Digenea: Plagiorchiida: Allocreadiidae: *Creptotrema* sp. (Fig. 2)

53.5 \pm 11.8, 33–73 mm TL, 29/44 (66%) overall, 10.1 \pm 14.3, range 1–68, 29/36 (81%) Bear Creek at Bear, Garland Co. (34.535034°N, 93.286517°W). 21 May, 2 Jul., 6 Sept., 16 Oct., 25 Nov., 2014, 20 May, 8 Jun., 2015, HWML 75384–86.

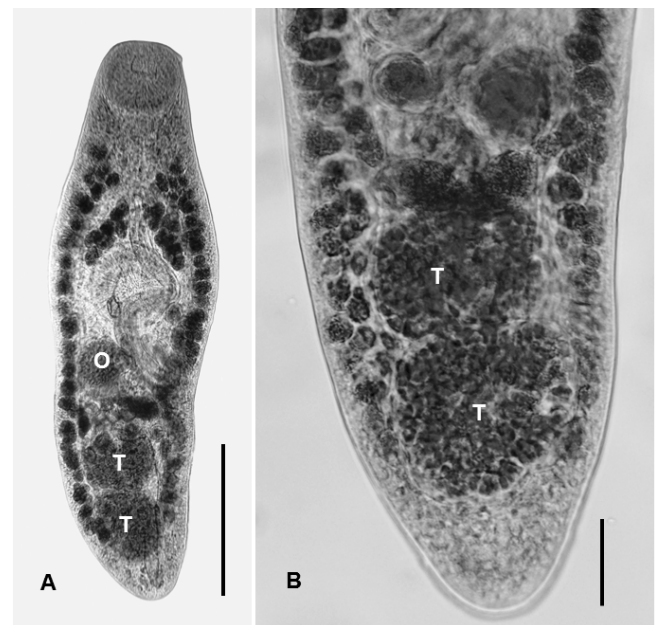


Figure 2. *Creptotrema* sp. from *Fundulus olivaceus*. A. Entire specimen showing ovary (O) and testes (T). Scale bar = 300 μ m. B. Higher magnification of another specimen showing irregular margins on testes (T). Scale bar = 100 μ m.

Interestingly, the highest intensity of infection of *Creptotrema* sp. was 68 adult and immature worms observed in a 63 mm TL specimen of *F. olivaceus* collected on 8 June 2015 from our Bear Creek site. In addition, one of the smallest juvenile *F. olivaceus* (33 mm TL) examined harbored 15 worms, also from Bear Creek.

Species of *Creptotrema* have been reported from esocids, fundulids, gasterosteids and percids in Arkansas, Florida, Mississippi, New York and Ohio, USA, and Manitoba, Nova Scotia and Ontario, Canada (Hoffman 1999, Curran et al. 2012, McAllister et al. 2016). Recently in Arkansas, McAllister et al. (2016) reported *Creptotrema* sp. from Northern Studfish, *Fundulus catenatus* from a stream within the Bear Creek watershed that cannot be morphologically

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differentiated from our specimens from *F. olivaceus*. Comparison of our specimens of *Creptotrema* sp. ($n = 10$) to the description of *Creptotrema funduli* Mueller, 1934 from Banded Killifish, *Fundulus diaphanus* from Oneida Lake, New York, show significant morphological differences as follows: (1) the testes in our specimens (Fig. 2A) have irregular margins versus those of Mueller's which have smooth margins; (2) *Creptotrema* sp. from Arkansas attain a greater body length (up to 1,300 μm as opposed to a maximum length of 1,000 μm in *C. funduli*); and (3) our specimens possess a longer cirrus sac representing 47–54% of body length, BL (cirrus sac measured along middle of sac throughout its length) with more pronounced coiling than that of *C. funduli* from New York that measures in length only 28% BL (Mueller's line drawing) (Mueller 1934). Our specimens most closely resemble those of the *C. funduli* reported by Curran et al. (2012) from *F. notatus* from Mississippi that also have similar testes and a long cirrus sac (42% of BL in line drawing of Curran et al. [2012]). However, *Creptotrema* sp. from Arkansas can be readily differentiated from both *C. funduli* from Mississippi and *C. funduli* from New York in having oral sucker-to-ventral sucker-width ratios ranging from 1:1.0–1.1 as opposed to those of *C. funduli* from Mississippi and New York that respectively range from 1:1.2–1.4 and from 1:1.2–1.5 (Curran et al. 2012). Therefore, we think that our *Creptotrema* sp. is new and will describe it in a forthcoming publication that will include comparison of rDNA sequences and tegument ultrastructure (through SEM) with congeners from North America. This digenean is reported for the second time from Arkansas and *F. olivaceus* is a new host.

Nematoda: Dioctophymatoidea: Dioctophymatidae: *Eustrongylides* sp. (4th stage larvae) (Fig. 3)

60 mm TL, 1/44 (2%) overall, 1/2 (50%) Boone Co., one worm, Crooked Creek at Harmon, Boone Co. (36.233894°N, 92.922276°W), 23 Jul., 2014, HWML 64766.

Eustrongylides spp. are found as adults in the proventriculus of piscivorous wading birds, with larvae encysted in the body cavity and musculature of fishes (Hoffman 1999). Specific identification of *Eustrongylides* requires rearing larvae in an avian host or DNA sequencing and our study did not include these techniques. This large, red-colored nematode (130 mm TL) was found encapsulated in the mesentery (Fig. 3A). McAllister et al. (2016) previously reported *Eustrongylides* from *F. catenatus* from Crooked Creek,

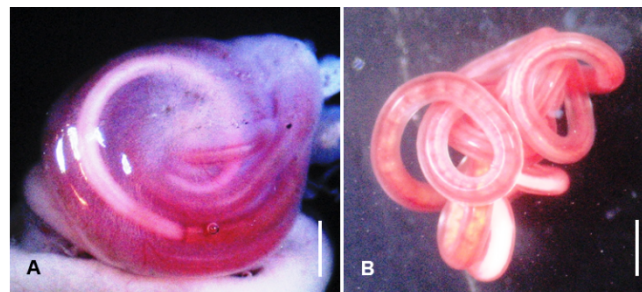


Figure 3. *Eustrongylides* sp. from *Fundulus olivaceus*. A. Encapsulated larvae from mesenteries. B. Individual worm teased from encapsulation. Scale bars = 2.5 mm.

Marion County. Therefore, we report, for the first time, *Eustrongylides* sp. from *F. olivaceus*.

Spirurida: Rhabdochonidae: *Rhabdochona cascadilla* Wigdor, 1918

55 mm TL, 1/44 (2%) overall, 1/2 (50%) Boone Co., two (one male, one female) worms, Crooked Creek at Harmon, Boone Co., (36.233894°N, 92.922276°W), 23 July 2014, HWML 64767.

In Arkansas, Cloutman (1976) reported *R. cascadilla* from Stonerollers, *Camptostoma* spp., from the White River, Washington Co., and, more recently, McAllister et al. (2016) found this nematode commonly in *F. catenatus*, from Crooked Creek, Marion Co. Intermediate hosts of *Rhabdochona* spp. are primarily mayflies but caddisflies and stoneflies also serve (Gustafson 1939, Barger and Janovy 1994, Moravec 1995). This is the second time *R. cascadilla* has been reported from any member of the family Fundulidae, and *F. olivaceus* is a new host. This nematode shows little host specificity as it has been previously reported from at least 38 genera within 13 families of freshwater fishes in Canada and the USA (see Hoffman 1999, Moravec 2007, 2010).

In conclusion, the information provided herein serves to supplement the known information regarding parasites of non-game fishes in Arkansas. With additional surveys, more new host and distributional records will be expected as well as description of new species.

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***Crepidostomum cornutum* (Digenea: Allocreadiidae) from Midget Crayfish, *Orconectes (Procericambarus) nana* (Decapoda: Cambaridae), from Northwestern Arkansas**

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Running Title: Trematode from Midget Crayfish

The midget crayfish, *Orconectes (Procericambarus) nana* Williams is a diminutive species (maximum length = 3.0 cm) that is found in the Neosho River Basin of northwest Arkansas and northeast Oklahoma (Reimer 1963, Hayes 1973, Morehouse and Tobler 2013). In addition, *O. nana* has been reported in the Illinois River (Bergey et al. 2005), and into the White River drainage (Prairie Creek) of Arkansas (C. Taylor, *pers. comm.*). In Arkansas, it has been collected from sites in Benton and Washington counties. The midget crayfish is found in clear-flowing, permanent small creeks and larger streams with substrate consisting of limestone gravel and cobble (Williams 1952, 1954). It was once thought to consist of two subspecies (Williams 1952, Hayes 1973) but a recent mtDNA study by Dillman et al. (2010) supported full species status originally suggested by Hobbs (1972) and Fitzpatrick (1987) for both *O. nana* and the Neosho midget crayfish, *O. macrus* Williams. This species' habitat is under constant threat from agriculture, road construction and urbanization, causing sedimentation and water pollution, in addition to construction of dams. Thus, this species has a State Heritage rank of S2 (imperiled) by NatureServe (2014) and has been assessed as vulnerable by the American Fisheries Society (Taylor et al. 2007).

Although information is available on the ecology of *O. nana* (see citations above), nothing, to our knowledge, has been published on any parasite of this crayfish. Here, we report a trematode parasite from *O. nana* from Arkansas.

Nine adult *O. nana* were collected on 19 November 2011 with a dipnet by overturning rocks at Flint Creek (Illinois River drainage) off St. Hwy. 59S, 0.5 km S of Gentry, Benton County (36.242699°N, 94.487472°W). In addition, on the same date, 7 adult *O. macrus* were taken with a dipnet from a tributary of Spavinaw Creek off St. Hwy 102, SE of Maysville, Benton County (36.364594°N, 94.551124°W).

Specimens were placed in aerated creek water and transported to the laboratory within 24 hr. They were killed by immersion in a concentrated Chloretone solution and their antennal glands, hepatopancreas and heart were placed in Petri dishes containing 0.6% w/v saline and examined for encysted parasites. Metacercariae were carefully teased from antennal gland cysts using insulin needles and fixed in hot ethanol, stained with acetocarmine and mounted in Canada balsam. Parasites were deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, and vouchers of crayfish were deposited in the Henderson State University (HSU) Collection, Arkadelphia.

None of the *O. macrus* were found to be infected, however, 2 of 9 (22%) *O. nana* were each found to be harboring one metacercaria (USNPC 105277–105278) in their antennal glands that fit the description of *Crepidostomum cornutum* (Osborn, 1903) Stafford, 1904, given by Cheng (1957). Morphological features of these 2 metacercariae are as follows: genital pore slightly posterior to cecal bifurcation; anterior extent of vitellaria at genital pore; posterior extent of vitellaria near posterior end; prostate in anterior one-half of cirrus sac; posterior extent of cirrus sac at anterior margin of anterior testis; prostate in anterior two-thirds of cirrus sac. Measurements are as follows (in µm, length × width): body, 1,872–2,541 × 474–498; oral sucker, 269–284 × 316–395; pharynx, 98–117 × 82; ventral sucker, 222–254 × 230–242; ovary, 144–148 × 117–137; anterior testis, 234–250 × 203–238; posterior testis, 226–242 × 211–234.

Crepidostomum cornutum has been reported previously in various crayfish (*Cambarus* and *Orconectes* spp.) from Illinois, Kansas, Louisiana, Michigan, Minnesota, Mississippi, New York, Oklahoma, Virginia, and Wisconsin, and Ontario and Quebec, Canada (Faust 1918, Hopkins 1934, Henderson 1938). The life cycle involves cercaria in

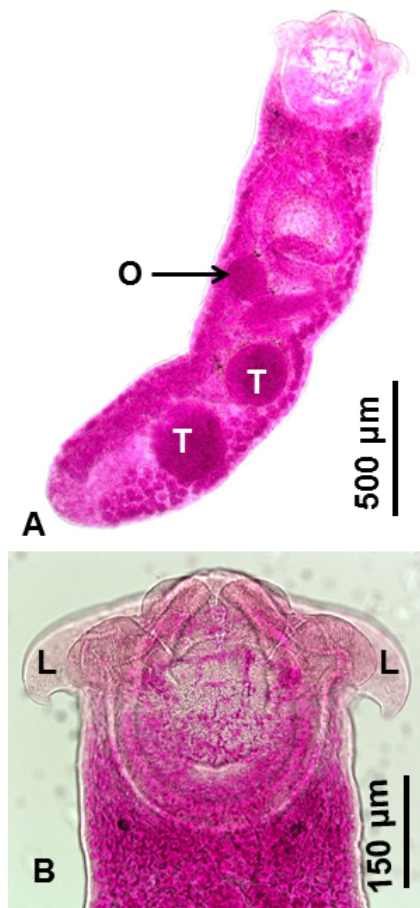


Figure 1. *Crepidostomum cornutum* metacercariae. A. Specimen showing ovary (O) and testes (T). B. Close-up of anterior end of specimen showing lappets (L) characteristic of the genus.

first intermediate freshwater bivalves (*Musculium* and *Sphaerium* spp.), metacercaria encyst in second intermediate host crayfishes, and adults are found in the pyloric ceca and intestinal tract of fish definitive hosts (Ameel 1937, Cheng 1957). There are many fishes reported as hosts including those in the genera *Ambloplites*, *Amia*, *Anguilla*, *Carassius* (experimental), *Gasterosteus*, *Ictalurus*, *Lepomis*, *Micropterus*, *Morone*, *Notemigonus*, *Perca*, *Pomoxis*, *Pylodictus*, *Salmo*, and *Salvelinus* (Hoffman 1999).

Previous reports of *C. cornutum* from Arkansas include specimens from game fishes, including Channel Catfish (*Ictalurus punctatus*), Warmouth (*Lepomis gulosus*), Bluegill (*Lepomis macrochirus*), Spotted Bass (*Micropterus punctulatus*), Largemouth Bass (*Micropterus salmoides*) and White Crappie (*Pomoxis annularis*) (Becker et al. 1966, Becker and Houghton 1969, Becker and Cloutman 1975, Cloutman 1975, Kilambi and Becker 1977). In addition, McAllister et al. (2014) recently reported *Crepidostomum cooperi* Hopkins, 1931 from Banded

Sculpin (*Cottus caroliniae*) from Flint Creek.

In summary, we document, to our knowledge, the first parasite ever reported from *O. nana*. Turner (1999) reported *Alloglossidium cardicolum* (Corkum and Turner) Smythe and Font from White River crayfish, *Procambarus acutus* from Arkansas. In addition, Turner (2006) reported *Allocorrigia filiformis* Turner and Corkum from red swamp crayfish, *Procambarus clarkii* from Arkansas, and Turner (2009) found *Alloglossidium dolandi* (Turner and McKeever) Smythe and Font in *P. acutus* from Arkansas. The only other previous report on Arkansas crayfish parasites was by McAllister et al. (2011) who reported metacercaria of *Alloglossidium corti* (Lamont) Van Cleave and Mueller from red-spotted stream crayfish, *Orconectes acares* and western painted crayfish, *Orconectes palmeri longimanus*. As Arkansas supports approximately 58 species of crayfishes, additional surveys should increase our knowledge of their parasites, of which we know very little about.

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A Noteworthy Geographic Distributional Record for the Milliped, *Apheloria virginiensis reducta* (Polydesmida: Xystodesmidae), from the Arkansas Delta

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Running Title: New Distribution Record for *Apheloria virginiensis reducta*

The milliped, *Apheloria virginiensis reducta* Chamberlin, 1939 is an attractive and colorful relatively large-bodied xystodesmid that ranges west of the Mississippi River from south of the Missouri River in central Missouri to extreme southeastern Kansas, the Interior Highlands of Arkansas, and further south to the far southeastern extremity of the Ouachita Physiographic Province in Oklahoma (Shelley and McAllister 2007, see their Fig. 2). In Arkansas, the reported distribution of *A. v. reducta* (Fig. 1) includes upland habitat in the Ouachita and Ozark Physiographic provinces with scattered records outside these provinces in four counties of Crowley's Ridge Physiographic Province in the far eastern part of the state (McAllister et al. 2002, 2003, 2013, Shelley and McAllister 2007). The type locality is Imboden, Lawrence County (Chamberlin 1939). Interestingly, Shelley and McAllister (2007) noted "...though Arkansas east of the Ouachitas has been poorly investigated, the milliped's absence from the heavily sampled adjoining corners of Texas, Arkansas, and Louisiana suggests that its absence from Coastal Plain areas to the north may be real." Therefore, given that no previous record of *A. v. reducta* has been reported from the Mississippi Alluvial Plain, we herein report a newly discovered population of this milliped from outside upland habitat in the Delta of far southeastern Arkansas.

On 28 June 2014 at 1600 hr, following moderate precipitation at an air temperature of 21°C, we collected 50 xystodesmids matching the description by Chamberlin (1939) of *A. v. reducta* from Pendleton Bend Park neighboring the Arkansas River, Desha County (33.987451°N, 91.362222°W). We also compared the gonopods of our specimens (Fig. 2 inset) to descriptions of those of *A. v. reducta* provided in Shelley (1978, his Figs. 65-66) and they possessed the diagnostic circular or "sickle-shaped" appearance. Habitat consisted of Arkansas River Valley shoreline

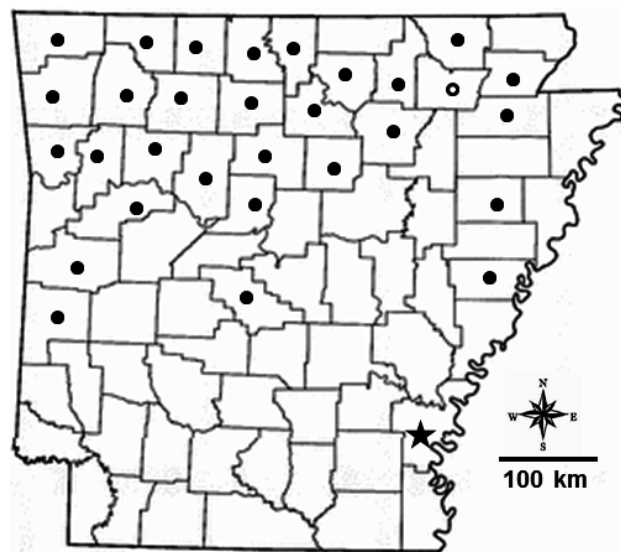


Figure 1. Previous Arkansas county records of *A. v. reducta* (dots), type locality (open dot) in Lawrence County and new record (star) in Desha County. For a complete geographic distribution in the U.S. see Shelley and McAllister (2007, their Fig. 1).

adjacent to a boat ramp. Five specimens were initially discovered under a trash can. Many others were photographed while traveling overland to retreats under rocks lining both sides of the boat ramp. Voucher specimens were placed in containers of 70% ethanol and select others were saved in DNA grade (95% v/v) ethanol. Voucher specimens were deposited in the Sam Noble Oklahoma Museum of Natural History, Oklahoma City, Oklahoma.

Numerous millipeds (estimated to be >500) were observed under and among concrete rock piles bordering the boat ramp. Only a few dead grasses and weeds were interspersed in this microhabitat which is unlike that ever reported for *A. v. reducta*. Previous reports (Shelley and McAllister 2007, McAllister et al. 2013) revealed that collections of *A. v. reducta* are typically made in upland deciduous forest with

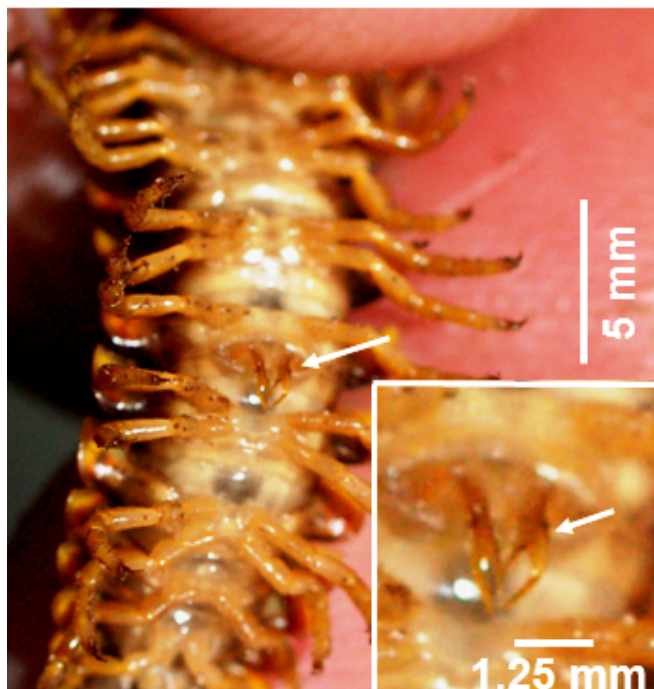
New Distribution Record for *Apheloria virginiensis reducta*

Figure 2. Male *A. v. reducta* showing gonopods (arrow). Inset: Higher magnification of left gonopod showing characteristic shape.

overstory dominated by oak (*Quercus* spp.). Specimens are usually taken from under decaying logs, small rocks, trash or other debris in areas with damp ground. These xystodesmids are known to squirt hydrogen cyanide from pores lining the sides of their body as a chemical defense, so care should be taken when collecting.

Although there was some variation among individuals, closer examination revealed the following: most possessed primarily yellow paranota and yellow transverse bands along the caudal metatergal margins with some semilunar splotches (see fig. 3C). Out of 40 individual adult *A. v. reducta* examined for gender, sex ratio was 2.3:1.0 (males: females).

In Arkansas, the previous most southeasterly located collection site for this millipede was along the southern periphery of Crowley's Ridge in Lee County at Bear Creek Lake Recreation Area (McAllister et al. 2013). Our new locality (Fig. 1) is over 100 km SSW of this location and situated geographically in the Mississippi Alluvial Plain physiographic region of the state. In addition, we did not observe additional *A. v. reducta* at two other boat ramps along this stretch of the river. The new site is also the southernmost locality in terms of latitude in the overall range of *A. v. reducta*. The previous southernmost locality was at Beavers Bend State Park in McCurtain County, Oklahoma (Shelley

and McAllister 2007). Finally, not only do we document a significant range extension but we also report the largest congregation, to our knowledge, of *A. v. reducta* ever reported from one locality.



Figure 3. Specimens of *A. v. reducta* observed at the study site. A. Groups of individuals (arrows) crawling overland. B. Two individuals seeking refuge under broken concrete boulders at Pendleton boat ramp. C. Single *A. v. reducta* showing ornamentation and coloration.

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The Arkansas Game and Fish Commission provided a Scientific Collecting Permit to CTM.

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New County Record of Black-Spot Disease in Arkansas

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Running Title: New County Record of Black-Spot Disease in Arkansas

Black-spot disease is an infection in fishes caused by metacercariae of neascus-type (*Uvulifer ambloplitis*, *Crassiphiala bulboglossa*, and others) and non-neascus type (*Apophallus brevis*, *Cryptocotyle lingua*, and others) digenetic trematodes (Hoffman 1999, McAllister et al. 2013, Roberts et al. 2013). Much of what is known about black-spot disease in Arkansas is from game species (Cloutman 1974, Becker and Cloutman 1975 and Hlass et al. 1998), although McAllister et al. (2013) provided several accounts of infection from new host species, both game and nongame, and new counties (Figure 1).

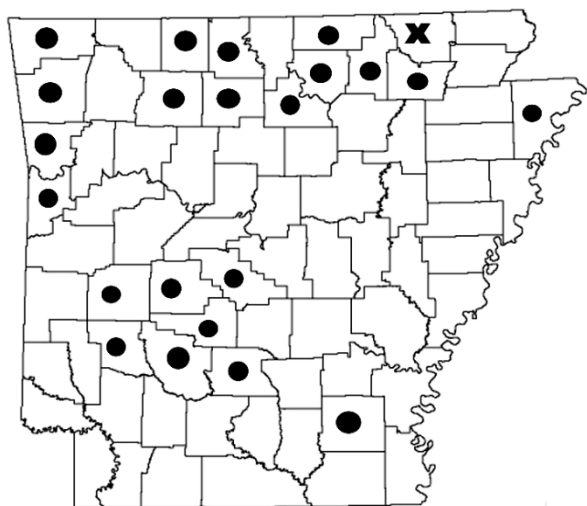


Figure 1. Counties where black-spot disease has been documented. Accounts by McAllister et al. (2013) (•) and reports herein (x).

As this report is intended to expand the knowledge of the distribution of black-spot disease in Arkansas, we did not assess prevalence of infection, infection abundance or intensity. Fish were collected from an upstream and downstream location from Jane's Creek in Randolph County on 17 January 2015. Fish were fixed in the field in a 10% neutral buffered formalin solution and placed on ice. The fish were then stored in 70% ethanol and held in the teaching collection.

Species was determined using *Fishes of Arkansas* (Robison and Buchanan 1988), and specimens were examined. Several species were infected with black-spot disease, including Central Stonerollers, *Camptostoma anomalum*, Bigeye Shiners, *Notropis boops*, a Telescope Shiner, *N. telescopus*, Greenside Darters, *Etheostoma blennioides*, Rainbow Darters, *E. caeruleum*, Orangethroat Darters, *E. spectabile*, and Banded Darters, *E. zonale*. Although these species are known to be hosts of the black-spot causing trematodes, the only account of infection in Randolph County was from a mention in a paper on the diversity of fishes in Jane's Creek (Fowler and Harp 1974) where some cyprinids were infected, though no host species were identified and no percids were infected. Figures 2 and 3 contain images of several individuals infected with black-spot disease.

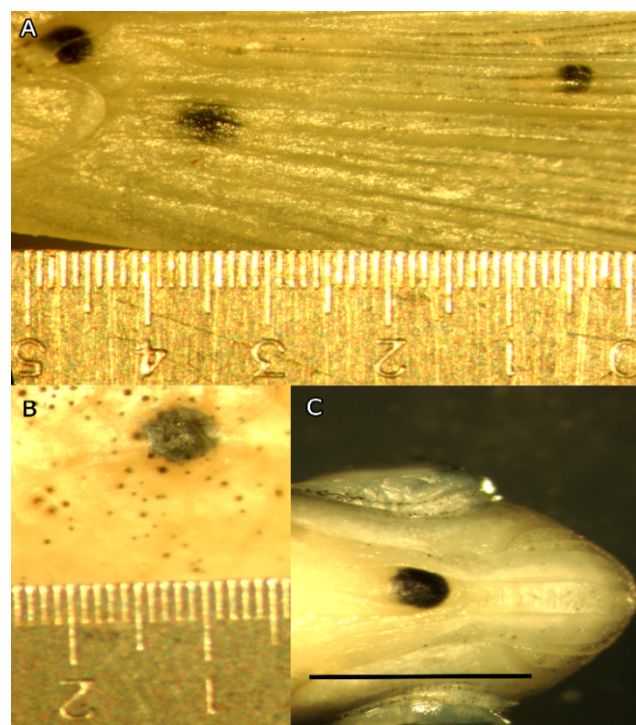


Figure 2. Encased cysts in caudal fin (A) and body (B) of a Central Stoneroller. Cyst on gular region of a Bigeye Shiner (C). Each notch on ruler is 100 μ m. Scale bar = 2 mm



Figure 3. Telescope Shiner (A), Bigeye Shiner (B), Central Stoneroller (C), Rainbow Darter (D), and Greenside Darter (E) infected with black-spot disease. Scale bar = 2 mm.

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We thank the Arkansas Game and Fish Commission for a scientific collection permit and Dr. Brook Fluker for assistance in fish identification.

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New Host and County Records of the Fish Leech *Cystobranchus klemmi* (Hirudinida: Piscicolidae) in Arkansas

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Running Title: New Host and County Records of *Cystobranchus klemmi*

Cystobranchus klemmi is a piscicolid fish leech first described by Williams and Bureson (2005) as *Gonimosobdella klemmi* and transferred to *Cystobranchus* by Williams and Bureson (2006). The first specimens were obtained from the Central Stoneroller, *Camptostoma anomalum*, and Largescale Stoneroller, *Camptostoma oligolepis*, from the Little Red River in Searcy County, Arkansas. Subsequently, Richardson et al. (2013) reported *C. klemmi* from Carroll, Franklin, Garland, Howard, Hot Spring, Independence, Madison, Montgomery, Pike, and Sevier counties in Arkansas (Fig. 1) and expanded the host list to include the Southern Redbelly Dace, *Chrosomus erythrogaster*, and Creek Chub, *Semotilus atromaculatus*.

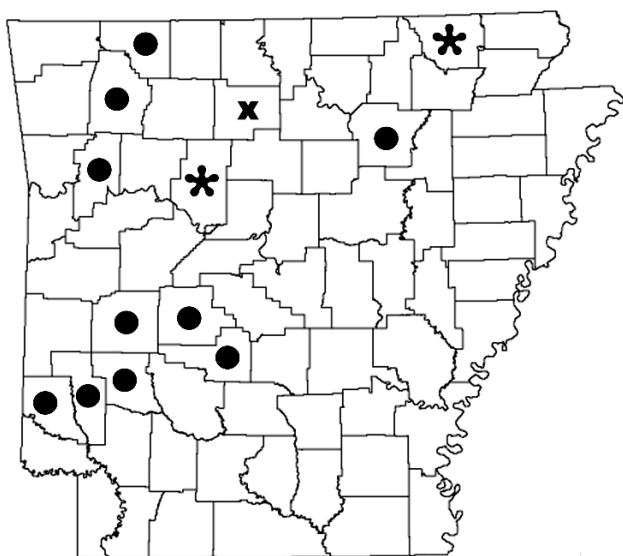


Figure 1. Known distribution of *Cystobranchus klemmi* as given by Williams and Bureson (2005) (X) and Richardson et al. (2013) (•). Localities reported herein (*).

A single individual of *C. klemmi* was collected from a Bigeye Shiner, *Notropis boops*, on 4 April 2014 from Eassis Creek in Randolph County (YPM67784) representing a new host and county record in Arkansas. Eighteen individuals *C. klemmi* were also collected from a single *C. anomalum* on 9 April 2014 from McCoy Creek in Pope County (YPM IZ 075985) representing a new county record. *C. klemmi* was also collected on 17 January 2015 from Central Stonerollers from Jane's Creek in Randolph County (YPM IZ 075986) (Fig. 2).

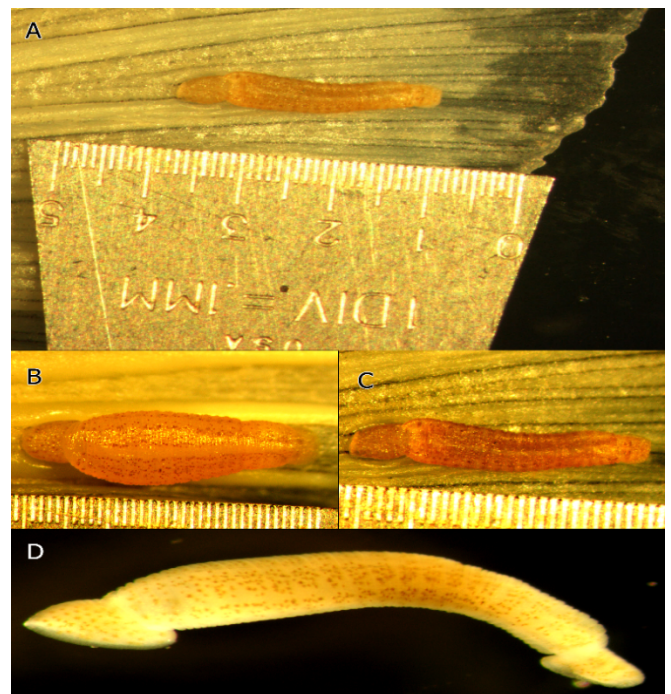


Figure 2. Leeches collected on 17 January 2015. Leech on caudal fin of *C. anomalum* (A, B, C) and detached leech (D). Each notch on ruler is 100 μm.

Voucher specimens of leeches were deposited in the Invertebrate Zoology Collections of the Peabody Museum of Natural History at Yale University, New Haven, Connecticut.

In summary, we have provided additional information on *C. klemmi* in Arkansas. Additional study is suggested in other parts of the state, particularly eastern and southern Arkansas, where other potential hosts of this leech might be expected.

Acknowledgments

We thank the Arkansas Game and Fish Commission for a scientific collection permit and Dr. Brook Fluker for assistance in fish identification.

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Recent History of Mountain Lion (*Puma concolor*) Observations in Arkansas, with Notes on the Individual Killed in Bradley County, Arkansas in 2014

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Running Title: Recent History of Mountain Lion (*Puma concolor*) Observations in Arkansas

Mountain lions (*Puma concolor*) were common into the 1800s in Arkansas but were believed to have been exterminated by the early 1900s, until an individual was killed in Montgomery County in 1949 (Sealander 1951) and a large male was killed after being treed by hounds near Mena (Polk County) the same year (Lewis 1969). For the next 20 years, numerous unverified sightings were reported around the state (Sealander 1956, Sealander and Gipson 1973) but without tangible support – most were reported as “reliable” observations by “qualified personnel”, but who, and how reliable, those reporters were was not documented. It was left to the reader to accept the statements without scientific credibility – until a specimen was killed in 1969 in Ashley County (Noble 1971). The last reported kill occurred 40 years ago (November 1975) in Logan County (McBride et al. 1993).

In 1978, steroid analysis of a scat confirmed the presence of another mountain lion in western Arkansas (Clark et al. 2002). Despite the numerous reported sightings of mountain lions over the years, field surveys conducted from 1988-1991 were unable to validate the presence of wild, reproducing populations of mountain lions in the state (McBride et al. 1993). Further, McBride et al. (1993) determined that all casts of tracks, scats, and photographs held by the Arkansas Game and Fish Commission (AGFC) were actually from coyotes, dogs, bobcats, or bears, and they argued that data used to identify sign of mountain lions in earlier publications were incorrectly interpreted.

However, based on scats and tracks, Witsell et al. (1999) later reported mountain lion activity along the eastern Ouachita Mountains in Garland, Hot Spring, and Pulaski counties. More recently, images have appeared on the internet purportedly taken by trail cameras set in Arkansas, but those often are of dubious origin or have not been verified.

Still, more scientific evidence has fostered the belief that mountain lions may be recolonizing former

range (Pike et al. 1997, LaRue et al., 2012) including Arkansas and neighboring states of Oklahoma, Missouri, and Louisiana. Williams (2015) noted confirmations of 4 mountain lions in Arkansas during the 5 years preceding the Bradley County kill, from Caney Creek WMA (southwest Arkansas), and the counties of Van Buren, Stone, and Marion in northern Arkansas. Three residents in Bella Vista (Benton County) reported seeing a mountain lion between 5-9 January 2015, but wildlife officers could not find sign or other tangible evidence to validate the observation (article published 16 January 2015 at www.arkansasonline.com/news/).

Even when sightings, images, tracks, or other signs of such wide-ranging mammals can be verified, biologists typically still do not know if the animal might be a transient dispersing from a distant viable population. For example, a subadult male mountain lion is known to have dispersed a straight-line distance of 1,067 km from the Black Hills of South Dakota and Wyoming to east-central Oklahoma (Thompson and Jenks 2005). Another male mountain lion killed near Milford, CT on 11 June 2011 was genetically tied to the Black Hills and matched an animal earlier located in Wisconsin and Minnesota, thus it had travelled about 2,400 km (New York Times, 27 July 2011, p. A17). Further, mountain lions kept as pets may be released or escape from captivity, and sightings of those can be misinterpreted as wild animals. In 1987, the AGFC reported a dead mountain lion in Franklin County (Clark et al. 2002), which likely had been a captive animal because it had been declawed. In 2001, up to 150 pet mountain lions were believed to be present in 20 Arkansas counties, and 8 escapes were known between 1997-2001 (Sasse 2001).

In Arkansas, it is illegal to kill a Mountain Lion unless it poses immediate danger to a person's life. To understand the current, and potentially changing, status of mountain lions in Arkansas, it is important to gain as much information as possible from any tangible

materials that become available. Previous reports of kills mention location of the kill but very little more (typically just length and weight), and only the Ashley County kill appears to be represented by a catalogued museum specimen (Noble 1971). Herein we report observations based on the carcass of the mountain lion killed in Arkansas during 2014.

Location of kill: On 8 November 2014, a deer hunter killed a male mountain lion in a hardwood area about 3.5 km (2.2 mi.) ESE of Hermitage, Bradley County, AR (GPS decimal degrees 33.441678°N, -92.135114°W). This location is only about 48 km (30 mi.) NW of the 1969 kill from 6 mi. (9.5 km) E of Hamburg, Ashley County (Noble 1971), and is near the Saline and Ouachita River bottomlands, considered by Sealander and Gipson (1973) to still be occupied by mountain lions at that time. Further, the area is near Felsenthal National Wildlife Refuge, which is suitable habitat for mountain lions (Thatcher et al. 2006, LaRue and Nielsen 2011), and in a rural area where mountain lions are more likely to occur (Pike et al. 1999).

Measurements and condition: The following measurements are presented in the units originally taken, with conversions added parenthetically to allow comparisons with other reports. The Bradley County male weighed 67 kg (148 lbs). Its total length was 222.3 cm (7 ft, 3.5 in.), tail length 74.9 cm (29.5 in.), head width 16 cm (6.3 in.), head length 28 cm (11.0 in.), and ear length 8 cm (3.2 in.).

Selected measurements (in mm) of the cleaned skull (Figure 1) included: total length, 215 mm (8.5 in.); zygomatic breadth, 150.2 mm (5.9 in.); height of sagittal crest, 9.9 mm (0.4 in.); length of right mandible, 141 mm (5.6 in.); and length of right upper canine, 37 mm (1.5 in.). For comparison, the Ashley County specimen weighed 69 kg (152 lbs) and was measured 210.8 cm (6 ft, 11 in.) in total length. Selected skull measurements were: total length, 212.7 mm (8.4 in.); zygomatic breadth, 144.4 mm (5.7 in.); height of sagittal crest, 9.5 mm (0.4 in.); length of right mandible, 140 mm (5.5 in.); and length of right upper canine, 29.4 mm (1.2 in.; Noble 1971).

The Montgomery County specimen killed in 1949 about 2 mi. (3.2 km) N Sims was 7 ft. long (about 2.1 m) and weighed 134 lbs (60.8 kg; Sealander 1951).

Fusion of epiphyses in all long bones indicated the specimen to be at least subadult. Weight and minimal tooth wear led us to an age estimate of 2-3 years old (Ashman 1983). Appearance of the muscle mass, appreciable deposits of fat in the omentum, and fat

surrounding organs indicated that this individual was in good physical condition.

The right scapula showed irregular osteological repair of old damage along the upper anterior curvature, indicating a likely hard blow to the right shoulder during the growth of the cat.

Reproductive condition: The testes were 35 mm long by 22 mm wide. A smear from the epididymis did not reveal spermatogenic activity, indicating that the specimen may not have achieved sexual maturity.

Foods: The stomach contained chicken bones but no feathers, which means the cat may have scavenged bones of a cooked chicken discarded by humans. However, meat of white-tailed deer (*Odocoileus virginianus*), generally viewed as the favored prey, (Pierce and Bleich 2003), distended the stomach and accounted for a mass of 2.9 kg (6.5 lbs).

Origin: Available evidence indicated that this cat was a dispersing wild animal. The cat had not been declawed, which would be indicative of captivity (though the presence of claws does not completely exclude this possibility). Recent occurrences of



Figure 1. Dorsal, lateral, and ventral views of the skull, and lateral view of the dentary, of the mountain lion killed in Bradley County, 8 November 2014.

Recent History of Mountain Lion (*Puma concolor*) Observations in Arkansas

cougars in the midwestern United States were believed to be largely due to dispersal of subadult males (LaRue et al. 2012), and Biek et al. (2006) and Thompson and Jenks (2010) also inferred male-biased dispersal to avoid inbreeding. The Bradley County specimen was a male, as was the cougar killed in Ashley County (Noble 1971), and the one reported killed near Mena in 1949 (Lewis 1969).

To evaluate genetic origin of the specimen, the AGFC sent a sample to the National Genomics Center for Wildlife and Fish Conservation (NGC), Missoula, MT, for DNA analysis. Historically, the endangered Florida subspecies (*Puma concolor coryi*) was thought to occur in Arkansas (Hall 1981, Sealander and Heidt 1990). That subspecies designation was 1 of 32 named forms based on analysis of osteological and pelage characteristics (Young and Goldman 1946), but recent genetic analysis has supported only 6 subspecies, with the North American form now designated *Puma concolor cougar* (Culver et al. 2000). Taxonomically speaking, the Florida population appears to be a genetically unique and disjunct population within the single North American subspecies (Culver and Schwartz 2011).

The Bradley County cougar had a mitochondrial DNA (mtDNA) haplotype "M" which is the most common North American haplotype (Culver et al. 2000, Culver and Schwartz 2011). A panel of 20 microsatellite loci for nuclear DNA was evaluated and compared to samples from populations in 12 other states. The populations with the highest probability of originating the Bradley County specimen was Wyoming (56.5% probability) and the Black Hills of South Dakota and Wyoming (35.5 % probability). This reflects a 92% probability of origin from populations that became viable in the 1990s and have become a primary source for post-2005 breeding populations (LaRue et al. 2012). Record dispersal distances by males from populations in Wyoming and South Dakota have been documented (Thompson and Jenks 2005, 2010). Importantly, the evidence indicates that this animal was not related to the Florida form, which was believed to have been the form historically present in Arkansas.

Interestingly, the NGC also had on file results of the genetic analysis of a hair sample collected near Pindall (Searcy County) in northern Arkansas on 20 September 2014. A goat had been killed and cached, and a game camera set up at the cache site captured images of a mountain lion on 18 September that apparently had returned to the kill. A search of the cache site revealed some hairs caught on briars, which

were sent to NGC for analysis.

That sample was from the same male mountain lion that was killed in Bradley County (the report stated that the probability of these 2 samples matching by random chance is 5.49×10^{-15} , and the probability that the same genetic profile would exist between siblings is 1.31×10^{-6}). Given the kill date of 8 November 2014 for this individual, it had covered a straight-line distance through Arkansas of 300 km (185 mi.) in 50 days. The genetic data and apparent direction of travel are consistent with the idea of a southeastward dispersing male from the Black Hills area.

In contrast, LaRue and Nielsen (2008) had argued that the most likely dispersal corridor to areas of suitable habitat in Oklahoma, Arkansas, and Missouri was from cougar populations in west Texas. Arkansas was believed to contain the most favorable habitat of several evaluated states for dispersing mountain lions (LaRue and Nielsen 2011). Sweanor et al. (2000) found that males dispersed farther, and that males were more likely to traverse large expanses of noncougar habitat while dispersing. Thompson and Jenks (2010) argued that such long dispersal was best explained by a mate procurement hypothesis in which subadult male cougars cross regions void of extant populations until they encounter a breeding population where they can establish residency.

Other observations: The specimen had been shot previously in the left neck and shoulder. We recovered 18 squirrel shot from just inside the skin. As no hematomas were present with the pellets and no holes were present on the outside of the skin, we conclude that the wound was old.

The skull, paws, and tail were retained by the AGFC for educational purposes. The postcranial skeleton, along with a section of skin, were retained in the Henderson State University collection of vertebrates (HSU 839).

Acknowledgments

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New Records and Life History Observations of the American Badger (*Taxidea taxus*) in Arkansas

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Running Title: Observations of American badgers (*Taxidea taxus*) in Arkansas

The first American badger (*Taxidea taxus*) reported from Arkansas was collected in 1964 from Washington County in northwestern Arkansas (Sealander and Forsyth 1966). A sight record in Franklin County (Sealander and Heidt 1990) was later supported by a specimen trapped near the Arkansas River (Cartwright and Heidt 1994). A roadkill specimen provided a disjunct record in Stone County, well eastward of the known distribution (Cartwright and Heidt 1994). Since 2003, several new observations revealed a reproductive population in northeastern Arkansas (Tumlison et al. 2012). Additional observations of Arkansas badgers have accumulated, permitting further documentation of new records and observations of their life history in the state. Two new specimens were catalogued into the collection of mammals at Henderson State University (HSU).

New Records of Distribution

Craighead County – Several records dating from 2003 were reported for Craighead County by Tumlison et al. (2012). Since then, Derek Vinson reported to us his extended observation of a badger in Valley View, SW of Jonesboro. A dog chased the badger from his yard and it escaped via a ditch and into a cow pasture. This observation was in 1985, the earliest date presently known for NE Arkansas, and about 18 years before the other documented cases were made.

Crawford County – We have an anecdotal account of a roadkill badger along AR St. Hwy 59 in western Crawford County during 2010. It had been brought to Cockrum's Taxidermy, Rudy, AR and retired Arkansas Game and Fish Commission (AGFC) wildlife officer David Wilson saw the specimen.

This anecdotal record is supported by photos and a specimen of a badger shot in a yard in Van Buren (approximate GPS 35.458°N, -94.364°W) on 15 June

2011. The head was mounted by Cockrum's Taxidermy. The location was 1.9 km (1.2 mi.) NE of the Arkansas River and 0.6 km (0.4 mi) S of U.S. I-40.

Crittenden County – Several recent observations of badgers have occurred between the town of Marion and Wapanocca National Wildlife Refuge (NWR). On 24 May 2013, county workers photographed 3 live juvenile badgers on Roseboro Island Road, reportedly about 3.2 km (2 mi.) N of its junction with U.S. Hwy 64 (Figure 1). Badgers also were observed near this location in 2007 (Tumlison et al. 2012). Presence of juveniles allow us to infer the second observation of reproduction in Arkansas, the first being from near Proctor, 18.7 km (11.6 mi) to the SE (Tumlison et al. 2012).

On 11 June 2014, a roadkill lactating female (though to be dead only a couple of hours) was found adjacent to a bean field along Roseboro Island Road, about 5.5 km (3.4 mi.) WNW of Marion (GPS 35.22627°N, -90.25420°W). The specimen was collected and prepared as skin and skeleton and catalogued as HSU 831. Standard measurements were: total length 700 mm, tail length 150 mm, hind foot length 90 mm, ear length 60 mm, weight 7.7 kg (17 lbs). Epiphyses of all bones were completely fused, and considerable tooth wear indicated she was an older adult.

Photographs taken at these sites were matched with images available via Google Earth Streetview at the GPS coordinates, which revealed this location to be exactly the same as the 2013 observation of juveniles on the same road. The roadkill specimen was collected by K. Harris, who returned to the location the following day and obtained images and a video of another badger (which appeared to be an adult, perhaps the male) that moved from the road into the bean field. A search of the area revealed a den beneath an oak tree located about 10 m (33 ft.) from the kill site.

From the image taken of the 3 juveniles, we

counted the number of broken center line marks between the badgers and the den on Roseboro Road, and by application of the Google Earth ruler function, were able to estimate that the 3 juveniles photographed on 24 May 2013 at this site were 90 m (295 ft.) from the den at the time of observation. We used the standard width of highway striping (4 in., or about 100 mm) as a reference to estimate the length of a juvenile badger that was broadside in the image, resulting in an estimated body length of 16 in. (ca. 400 mm). The adult male and female specimens collected during this study had body lengths of 615 mm (24.2 in.) and 550 mm (21.6 in.), respectively. From this, we estimate that the juvenile badgers were 65% - 73% grown on 24 May.

On 15 October 2013, Bill Petersen photographed a roadkill badger at the edge of a cotton field along AR St. Hwy 77, about 1.1 km (0.7 mi.) N of Clarkdale (GPS 35.31905°N, -90.23970°W). Almost a year later, on 18 August 2014, he photo-documented another roadkill badger at the edge of a corn field about 1.6 km (1 mi.) W of this site, along U.S. I-55 (GPS 35.322564°N, -90.259220°W). These new records extend observations about 10.5 km (6.5 mi.) N of earlier records in the county (Tumilson et al. 2012).

Mississippi County – On 21 August 2014, a male badger was collected by C. Vlautin on the S shoulder of AR St. Hwy 18 near Manila, about 1.6 km (1 mi.) W of Big Lake NWR, (GPS 35.872112°N, -90.156273°W). The specimen was prepared as skin and skeleton, and catalogued as HSU 832. Standard measurements were: total length 750 mm, tail length 135 mm, hind foot length 105 mm, ear length 64 mm, weight 9.5 kg (21 lbs). Epiphyses of all bones were thoroughly fused, but tooth wear was light, indicating a young adult individual. The carcass appeared to be fresh with little external damage, although necropsy revealed broken bones and ruptured viscera, supporting the likelihood of road mortality as the cause of death. The specimen was nearly hairless over a dorsal oval extending from the neck to the tail, which appeared to have existed well before its death. We conjecture that such damage may have been caused by repeated abrasion during entrance and exit of a den. These abrasions were inconsistent with patterns normally associated with mite infestations or other possible etiologies. This specimen is a new county record for Arkansas.

A roadkill on AR St. Hwy 119 E of Marie, Sec. 6, T11N, R10E was reported 19 January 2014 by R. Miller. This record is about 30 km (18.7 mi.) SSE of

the specimen record from near Manila and 35.5 km (22 mi.) NE of the nearest record in Crittenden County. No confirming evidence (e.g., an image) was available.

Sebastian County – A live badger was photographed under a parked car at 100 S 10th Street, Fort Smith on 1 May 2014, about 1.1 km (0.7 mi.) SE of the Arkansas River. The animal was captured by Fort Smith Police Dept. personnel and released at 300 Parker Ave, near the Arkansas River. This is a photo-vouchered new county record and apparently the first documented Arkansas observation S of the Arkansas River since the 1983 capture of a male badger 2.5 km (1.6 mi.) S of the Ozark Dam on the Arkansas River, Franklin County (Cartwright and Heidt 1994). This occurrence is not surprising as badgers have been documented in neighboring LeFlore and McCurtain counties of Oklahoma (Tumilson and Bastarache 2007). The LeFlore County record was taken just S of the Kerr Lock and Dam on the Arkansas River, about 35 km (22 mi.) from this new Arkansas observation

The 2011 Crawford County record noted previously was located only 11.1 km (6.3 mi.) NE of the new Sebastian County Record, but on the N side of the Arkansas River. It is likely that the Arkansas River serves as a corridor for badgers dispersing into Arkansas from the west.

Dens

In 2009, trail camera images revealed a presumed coyote den to actually be the den of a family of badgers near Proctor, Crittenden County (Tumilson et al. 2012). This den faced a road and had excavated dirt deposited in front of the opening, creating a small mound.

The entrance to the den located on Roseboro Island Road in Crittenden County was positioned at the top of the incline from the road ditch, facing the road, and was somewhat protected by surrounding dead branches. Excavated dirt was deposited downslope of the entrance.

We have no observations of dens beyond the appearance of the entrances. Both of these were natal dens, each producing offspring. Deposition of excavated dirt at the entrance is typical of the species, and natal dens tend to be more complex than resting dens due to the amount of dirt excavated (Lindzey 2003). Badgers are known to move young among dens, and to use several different dens – some only for resting (Messick and Hornocker 1981). The nature of dens in northeastern Arkansas may be different than

Observations of American badgers (*Taxidea taxus*) in Arkansas

those reported for western populations, as agricultural practices in Arkansas may create a different landscape than western rangelands. For example, the female from Roseboro Island Road appears to have used the same natal den during consecutive years (based on 2013 and 2014 observations at the same site). Currently, these are the only observations and descriptions of badger dens in Arkansas.

In lowland areas subject to flooding, elevated den entrances should be advantageous. Higher elevations for dens also were selected by badgers in cropland of Illinois and Ohio (Duquette et al. 2014). In the Midwest, badgers were found to avoid roads (Duquette et al. 2014) yet our observations of dens (as well as distribution) were along roads. Data from Arkansas so far have been limited to chance observations along roads, therefore a detailed study is needed to understand space use of badgers in Arkansas croplands.

Litter size

Very little is known about biology of badgers in Arkansas, and reproduction in particular. Two observations in Crittenden County allow inferences of likely litter size. The observation of a den near Proctor on 29 May 2009 revealed 5 badgers, all of which were similar-sized (Tumilson et al. 2012). Because badgers do not den together except as family groups (Lindzey 2003), and these individuals were about mature, it is likely that they represent a successful litter of three or four (assuming one was the mother, leaving a litter of 3 if the paternal male was also present, or 4 if he was not).

The 24 May 2013 observations photodocumented a litter size of at least 3, as all individuals were clustered at the time of the photograph and away from the den.

Subspecies

Long (1972) treated the only badger specimen known at the time from Arkansas as *Taxidea taxus berlandieri*, although he also stated that specimens from eastern Kansas, southern Oklahoma, and northern Arkansas were intergrades of *T. t. berlandieri* and the more northern *T. t. taxus*. His treatment of the single Arkansas specimen was due to its small reported size, although only a skin was available (Sealander and Forsyth 1966) and it was not examined. Specimens from E Kansas also were considered to be intergrades, but were included as *T. t. taxus* in the distributional map of subspecies. Further, specimens from northern

and western Missouri were not examined, but Long (1972) attributed them to *T. t. taxus* based on location. Tumilson et al. (2012) believed that the population in northeastern Arkansas represented expansion of Missouri populations, therefore these could be considered *T. t. taxus*, but intergradation clouds the issue. We examined available materials to determine if subspecific identification could reasonably be assigned.

Long (1972) described the southern badger, *T. taxus berlandieri* as having a long mid-dorsal stripe, typically extending at least mid-dorsum, but usually to the rump. This form is small, has reddish pelage, and the sagittal ridges often do not merge dorsally. Photovouchers allowed us to examine the mid-dorsal stripe of 15 individuals from Arkansas. In all cases, the stripe ended no further caudally than the mid-scapular region, thus affiliating best with *T. taxus taxus*. However, coloration of the specimen (HSU 831) matched the description of *T. taxus berlandieri*, as did about half of the photographed individuals. Long (1972) considered the badgers in adjacent Oklahoma and Missouri to represent intergrades, and those are the source populations for the Arkansas dispersers.

The sagittal ridges of HSU 831 and 832 were merged dorsally, not supporting identification as *T. taxus berlandieri*, although the idea of intergradation cannot be dismissed. Specimens from NE Arkansas average looking more like *T. taxus taxus*. In any case, it is apparent that the Arkansas population represents expansion from 2 different sources, along the Arkansas River in W Arkansas and from SE Missouri in NE Arkansas.

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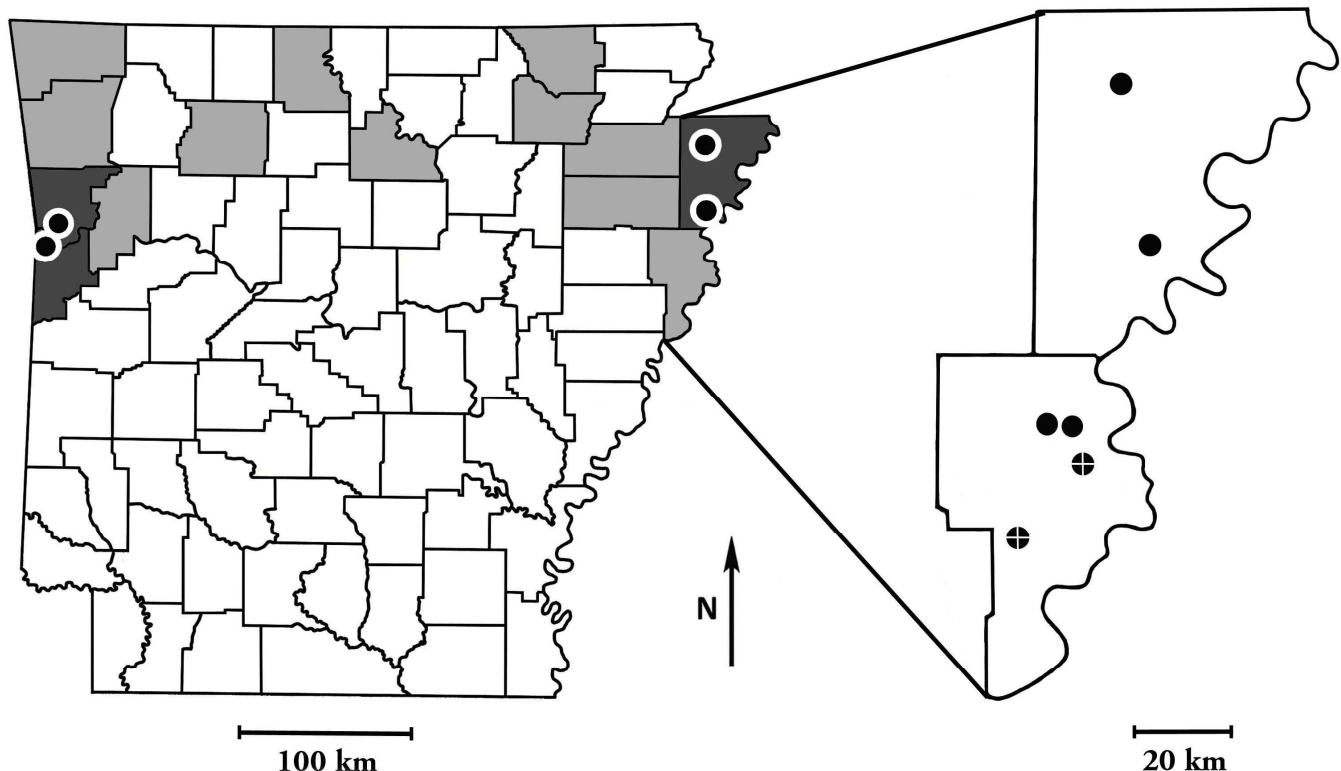


Figure 1. Currently known distribution of American badgers (*Taxidea taxus*) in Arkansas. Lighter shading shows counties with previously reported records. Darker shading indicates new county records, and dots within them show approximate location of records. Enlargement is of Mississippi County (top) and Crittenden County (bottom), with black dots indicating approximate locations of new records. Crossed dots show historic and new records of breeding.

Atypical Head Markings of the Ouachita Map Turtle (*Graptemys ouachitensis*) in the Upper Ouachita River of Arkansas

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Running title: Head Markings of Ouachita Map Turtle

Turtles of a clade historically known as false map turtles (*Graptemys pseudogeographica*) occur throughout the Mississippi River drainage, but phenotypic variation throughout their range has precipitated taxonomic confusion since their original description (Lindeman 2003). Currently, two forms are known in Arkansas and both occur statewide and often in the same body of water.

Graptemys pseudogeographica kohnii (Mississippi Map Turtle) is the designation for a form that possesses a yellowish crescent that originates dorsally behind each eye then descends laterally and curves forward, terminating in a position below the back of the eye. The crescent is comprised of the connection between markings located behind the eye (postorbital) and under the eye (subocular or supramandibular). Connection of these marks creates a barrier that prevents any other of the yellow head stripes from reaching the eye, and this characteristic was used as a diagnostic device to identify most specimens of this southern subspecies.

This crescented form was described originally as a unique species (Carr 1949). However, Vogt (1993) lowered its status from species to subspecies. Analysis of mitochondrial DNA (mtDNA) found no differences within *G. pseudogeographica*, including *G. p. kohnii* (Lamb et al. 1994), supporting Vogt's view. Lindeman (2003) noted that the taxonomic changes were not universally accepted.

A very similar form, the Ouachita Map turtle was described originally as a subspecies, *G. pseudogeographica ouachitensis* (Cagle 1953). However, Vogt (1993) considered *G. ouachitensis* to be a distinct species, and analysis of mtDNA demonstrated differences between *G. pseudogeographica* and *G. ouachitensis*, also supporting their distinction (Lamb et al. 1994). The postorbital, subocular, and mandibular (located at the back of the lower jaw) marks tend to be independent so appear as three distinct dots in *Graptemys ouachitensis* – at least in southern populations – which allows 1-3

lines from the neck to reach the orbit, and this has been a primary characteristic used in keys to aid in identification of this form (Trauth et al. 2004, Ernst and Lovich 2009). However, northern populations of *G. ouachitensis* tend to have the postorbital and subocular spots widely joined, though the resulting bar is wider than in *G. pseudogeographica kohnii* (Vogt 1993, Lindeman 2003, 2013).

Potential identification of *Graptemys* species is further confounded by the observation that some of the species in the genus can hybridize in sympatry (Godwin et al. 2014, Lindeman 2003), and the primary isolating mechanism preventing hybridization may be allopatry (Godwin et al. 2014). Still, Vogt (1993) had argued that head markings likely were important for species recognition during courtship, and Lindeman (2003) believed that use of combinations of characters would allow accurate discrimination of these taxa.

We collected 15 juvenile *G. ouachitensis* and 5 juvenile *G. pseudogeographica kohnii* syntopically from Lake Hamilton and the Ouachita River in Clark and Garland counties during 2014, and compared characteristics with available literature. Because most of our specimens of *G. ouachitensis* did not conform to written descriptions for southern populations (but did more so for northern populations), we followed Lindeman's (2003) approach for discrimination. Here, we propose some new means of discrimination in Arkansas, particularly adding considerations regarding juvenile characteristics not previously available in the literature.

Via examination of juvenile specimens, we found differences between these taxa based on coloration and shape of head stripes, eye coloration, and degree of pigmentation of the plastron. Further, large yellow markings on the chin tended to form a chin bar on *G. ouachitensis* but were only small spots on *G. p. kohnii*, similar to the differences found between *G. barbouri* and *G. ernsti* (Godwin et al. 2014). Lindeman (2013) did note large chin spots in *G. ouachitensis*, but did not report examples of the spots joining to form chin bars.

The following group of characteristics, taken together, should allow distinction of specimens of either species in Arkansas (particularly if juveniles are available). For comparisons, see Figures 1 and 2. It should be noted that Lindeman (2003) found many of these same characters in populations of both forms in Kentucky Lake, and suggested that several characters taken together should lead to accurate identification, although there is much variation seen in coloration patterns. However, characters seen in juveniles, but

that disappear during ontogeny, have not been discussed and compared between these species previously in the literature for southern populations. For example, Ernst and Lovich's (2009) summary of literature noted that plastron patterns of juvenile *G. ouachitensis* fade with age, and they noted characters of hatchling *G. pseudogeographica kohnii* from Wisconsin, but no comparisons of juvenile traits were given.



Figure 1. Dorsal, lateral, and ventral views of the head, and views of the plastron of juveniles of *Graptemys pseudogeographica kohnii*.

Head Markings of Ouachita Map Turtle



Figure 2. Dorsal, lateral, and ventral views of the head, and views of the plastron of juveniles of *Graptemys ouachitensis*.

1a. Iris white, lacking any black stripe; a light crescent around the back of the eye prevents any yellow lines on neck from reaching the margin of the eye (crescent is about the width of the pupil of the eye); crescent terminates under the eye; crescent orange with lighter border in juveniles but may fade to yellow in adults; ventral chin markings most commonly with 3 small orange or yellow spots (1 central and 2 near angles of jaw, Figure 1); plastron of juveniles more extensively pigmented with thick lines (plastral pigment diffuses with age)*Graptemys pseudogeographica kohnii*.

1b. Black stripe, or at least a line of black pigment flecks, present in iris; postorbital and subocular spots separate, allowing yellow lines on neck to reach the margin of the eye, or if these spots are joined the resulting crescent is irregular in shape and about twice as wide as the pupil of the eye; postorbital and subocular spots straw yellow whether joined or distinct; if crescent present, it underscores the eye and joins with another stripe, terminating on the snout; ventral chin markings most commonly with 3 large spots that usually coalesce to form a wedge-shaped bar (Figure 2); plastron of juveniles less extensively pigmented with narrow lines (plastral pigment diffuses with age)*Graptemys ouachitensis*.

Specimens used in this study were photovouchered (as presented in the figures) and a few specimens were catalogued into the Henderson State University collection of vertebrates: *G. pseudogeographica kohnii* HSU 1746 – 1748 and *G. ouachitensis* HSU 1731 – 1733. Carapace lengths of specimens examined were less than 50 mm.

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The prompt return of revised manuscripts and payment of publication costs is critical for processing of the *JOURNAL* by the *JOURNAL* staff. If the corresponding author will be unable to attend to the manuscript within the framework of this schedule, then it is the responsibility of the corresponding author to make arrangements with a coauthor to handle the manuscript. NB. The corresponding author will be responsible for submitting the total publication cost of the paper by August 31st. Failure to pay the publication charges by the deadline will prevent processing of the manuscript, and the manuscript will be added to the manuscripts received from the following year's meeting.

Preparation of the Manuscript

A. General considerations

Format the manuscript as a published paper. If you are unfamiliar with the Journal, please access last year's journal at <http://libinfo.uark.edu/aas/>. to familiarize yourself with the layout.

1. Use Microsoft Word 2007 or higher for preparation of the document and the file should be saved as a Word Document.
2. The text should be single spaced with Top and Bottom margins set at 0.9" Left and Right margins, 0.6". Except for the Title section, the manuscript must be submitted in two column format and the distance between columns should be 0.5". This can be performed in Word 2007 by clicking on "Page layout" on the Toolbar and then "Columns" from the drop-down menu. Then select "two" (columns).
3. Indent paragraphs and subheadings 0.25".
4. Use 11 point font in Times New Roman for text. Fonts for the rest of the manuscript must be
 - a) Title: 14 point, bold, centered, followed by a single 12 point blank line.
 - b) Authors' names: 12 point, normal, centered. Single line spaced. Separate last author line from authors' address by a single 10 point blank line.
 - c) Authors' addresses: 10 point, italic, centered. Single line spaced. Separate last author line from corresponding author's email by a single 10 pt blank line.
 - d) Corresponding authors email: 10 point, normal,

left alignment.

- e) Running title: 10 point, normal, left alignment.
 - f) Main text: 11 point, justified left and right.
 - g) Figure captions: 9 point, normal.
 - h) Table captions: 11 point normal.
 - i) Section headings: 11 point, bold, flush left on a separate line, then insert an 11 pt line space. Section headings are not numbered.
 - j) Subheadings: 11 point, bold, italic and flush left on a separate line.
6. Set words in italics that are to be printed in italics (e.g., scientific names).
 7. In scientific text, **Arabic numerals** should be used in preference to words when the number designates anything that can be counted or measured: 3 hypotheses, 7 samples, 20 milligrams. However, numerals are not used to begin a sentence; spell out the number, reword the sentence, or join it to a previous sentence. Also, 2 numeric expressions should not be placed next to each other in a sentence. The pronoun "one" is always spelled out.
 8. A **feature article** is 2 or more pages in length. Most **feature articles** should include the following sections: Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgments, and Literature Cited.
 9. A **general note** is generally shorter, usually 1 to 2 pages and rarely utilizes subheadings. A note should have the title at the top of the first page with the body of the paper following. Abstracts are not used for general notes.
 10. A **review article** should contain a short abstract followed by the body of the paper. The article may be divided into sections if appropriate, and a final summary or concluding paragraph should be included.

B. Specific considerations

1. Title section

(see Fig. 1 on the next page for layout).

- i. It is important that the title be short, but informative. If specialized acronyms or abbreviations are used, the name/term should be first indicated in full followed by the short form/acronym.
- ii. Names of all authors and their complete mailing addresses should be added under the Title. Authors names should be in the form "A.M. Scientist", e.g. I.H. Still. Indicate which author is the corresponding author by an asterix, and then

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Title of a Paper (14 pt, bold, centered)

A.E. Firstauthor^{1*}, B.F. Second¹, C.G. Third², and D.H. Lastauthor¹ (12 point font, normal, centered)

¹*Department of Biology, Henderson State University, Arkadelphia, AR 71999*

²*Arkansas Game and Fish Commission, 915 E. Sevier Street, Benton, AR 72015 (10 point font, italic, centered)*

*Correspondence: Email address of the corresponding author (10 point, normal, left alignment)

Running title: (no more than 95 characters and spaces) (10 point, normal, left alignment)

Figure 1. Layout of the title section for a submitted manuscript.

indicate that author's email address on a separate line (see A.4 for format.)

iii. Please include a Short Informative **Running title** (not to exceed 95 characters and spaces) that the Managing editor can insert in the header of each odd numbered page.

iv. Insert a single 10pt blank line after the "Running Title" and add a Continuous section break.

2. Abstract

An **abstract** summarizing in concrete terms the methods, findings, and implications discussed in the body of the paper must accompany a **feature article** (or a **review article**). That abstract should be completely self-explanatory. A short summary abstract should also be included for any review article. Please review your title and abstract carefully to make sure they convey your essential points succinctly and clearly.

3. Materials and Methods

Sufficient details should be included for readers to repeat the experiment. Where possible reference any standard methods, or methods that have been used in previously published papers. Where kits have been used, methods are not required: include the manufacturer's name and location in brackets e.g. "RNA was prepared using the RNeasy Plus Micro Kit (Qiagen, USA)."

4. **Tables and figures** (line drawings, graphs, or black and white photographs) should not repeat data contained in the text. Tables, figures, graphs, pictures, etc. have to be inserted into the manuscript with "text wrapping" set as "top and bottom" (not "in line with text"). In the event that a table, a figure, or a photograph requires larger space than a single column, the two column format should be ended and the Table/figure should be

placed immediately afterward. The two column format should continue immediately after the Table/figure.

Tables and figures must be numbered, and should have titles and legends containing sufficient detail to make them easily understood. Allow two 9 pt line spaces above and below figures/tables. Please note that Figure and Table captions should be placed in the body of the manuscript text AND NOT in a text box.

i. **Tables:** A short caption in 11 point normal should be included. Insert a solid 1.5 pt line below the caption and at the bottom of the table. Within tables place a 0.75pt line under table headings or other divisions. Should the table continue to another page, do not place a line at the bottom of the table. On the next page, place the heading again with a 0.75pt line below, then a 1.5 pt line at the start of the table on the continued page. Tables can be inserted as Tables from Excel, but should not be inserted as pictures from Powerpoint, Photoshop etc., or from a specialized program.

ii. **Figures:** A short caption should be written under each figure in 9 point, normal. Figure 2 shows an example for the format of a figure inserted into the manuscript. All figures should be created with applications that are capable of preparing high-resolution PhotoShop compatible files. The figure should be appropriately sized and cropped to fit into either one or two columns. Figures should be inserted as JPEG, TIFF images or PhotoShop compatible files. While the Journal is printed in black and white, we encourage the inclusion of color figures and photographs that can be viewed in the online version. Please note that the figures directly imported from PowerPoint frequently show poor color, font and resolution issues.

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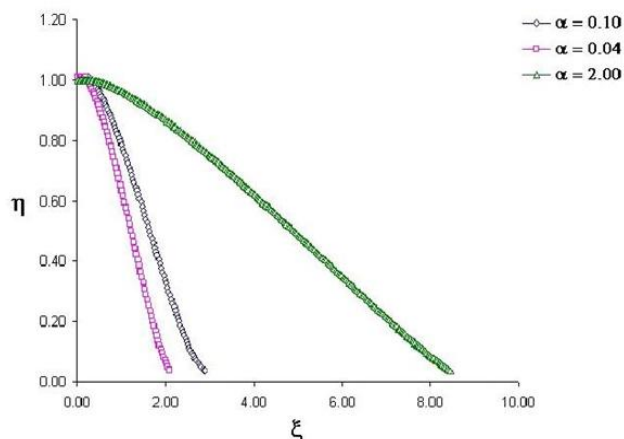


Figure 2. Electric field, η , as a function of position ξ , within the sheath region for three different wave speeds, α .

Figures generated in Powerpoint should be converted to a high resolution TIFF or JPEG file (see your software user's manual for details).

5. Chemical and mathematical usage

- i. The Journal recommends the use of the International System of Units (SI). The **metric system of measurements and weights** must be employed. **Grams** and **Kilograms** are units of **mass** not weight. Non-SI distance measurements are permitted in parentheses.
- ii. Numerical data should be reported with the number of significant figures that reflects the magnitude of experimental uncertainty.
- iii. Chemical equations, structural formulas and mathematical equations should be placed between successive lines of text. Equation numbers must be in parentheses and placed flush with right-hand margin of the column.

6. Deposition of materials and sequences in publicly available domains

Cataloguing and deposition of biological specimens into collections is expected. Publication of manuscripts will be contingent on a declaration that database accession numbers and/or voucher specimens will be made available to interested researchers. Where possible, collector and voucher number for each specimen should be stated in the Results section. The location of the collection should be stated in the Methods section. This will facilitate easy access should another researcher wish to obtain and examine the specimen in question.

7. Literature Cited

- i. Authors should use the Name – Year format as illustrated in *The CBE Manual for Authors, Editors, and Publishers* and as shown below. The *JOURNAL* will deviate from the form given in the *CBE Manual* only in regard to placement of authors' initials and abbreviation of journal titles. Initials for second and following authors will continue to be placed before the author's surname. Note that authors' names are in bold, single spacing occurs after periods. If a citation has 9 authors or more, write out the first 7 and append with *et al.* in the Literature Cited section. **Journal titles should be written in full.** Formats for a journal article and a book are shown below along with examples.
- ii. Please note how the literature is “cited in text as”, i.e. in the introduction, results etc. In general, cite in text by “first author et al.” followed by publication date. **DO NOT USE NUMBERS, etc.** Also note that in the Literature Cited section, references should be single line spaced, justified with second and following lines indented 0.25". Column break a reference in Literature Cited that runs into the next column so that the entire reference is together. Insert a Continuous Section break at the end of the Literature cited section.

Accuracy in referencing current literature is paramount. Authors are encouraged to use a reference databasing system such as Reference Manager or Endnote to enhance accurate citation. Do not cite abstracts and oral, unpublished presentations. Unnecessary referencing of the authors own work is discouraged; where possible the most recent reference should be quoted and appended with “and references therein”.

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Author(s). Year. Article Title. Journal title volume number(issue number):inclusive pages.

Author(s) [or editor(s)]. Year. Title of Book. Place of publication: publisher name. Number of pages.

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Standard Journal Article

Davis DH. 1993. Rhythmic activity in the short-tailed vole, *Microtus*. *Journal of Animal Ecology* 2:232-8
Cited in text as: (Davis 1993)

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Steiner U, JE Klein and LJ Fletters. 1992. Complete wetting from polymer mixtures. *Science* 258(5080):1122-9.

Cited in text as: (Steiner et al. 1992)

Zheng YF and JYS Luh. 1989. Optimal load distribution for two industrial robots handling a single object. *ASME Journal of Dynamic System, Measurement, and Control* 111:232-7.

Cited in text as: (Zheng and Luh 1989)

In press articles

Author(s). Expected publication Year. Article Title. Journal title *in press*.

Cited in text as: (First author et al. *in press*)

Kulawiec M, A Safina, MM Desouki, IH Still, S-I Matsui, A Bakin and KK Singh. 2008. Tumorigenic transformation of human breast epithelial cells induced by mitochondrial DNA depletion. *Cancer Biology & Therapy* *in press*.

Cited in text as: (Kulawiec et al. *in press*)

Books, Pamphlets, and Brochures

Box GEP, WG Hunter and JS Hunter. 1978. Statistics for experiments. J Wiley (NY). 653 p.

Cited in text as: (Box et al. 1978)

Gilman AG, TW Rall, AS Nies and P Taylor, editors. 1990. The pharmacological basis of therapeutics. 8th ed. Pergamon (NY). 1811 p.

Cited in text as: (Gilman et al. 1990)

Engelberger JF. 1989. Robotics in Service. MIT Press Cambridge (MA). 65p.

Cited in text as: (Engelberger 1989)

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Author(s) or editor(s). Year. Title of book. Publisher's name (Place of publication). Kind of part and its numeration, title of part; pages of part.

Hebel R and MW Stromberg. 1987. Anatomy of the laboratory cat. Williams & Wilkins (Baltimore). Part D, Nervous system; p 55-65.

Singleton S and BC Bennett. 1997. Handbook of microbiology. 2nd ed. Emmaus (Rodale, PA). Chapter 5, Engineering plasmids; p 285-96.

Book Chapter or Other Part with Different Authors

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Author(s) of the part. Year. Title of the part. *In* author(s) or editor(s) of the book. Title of the book. Publisher (Place of publication). Pages of the part.

Weins JA. 1996. Wildlife in patchy environments: Metapopulations, mosaics, and management. *In*: McCullough DR, editor. Metapopulations and wildlife conservation. Island Press (Washington, DC). p 506.

Johnson RC and RL Smith. 1985. Evaluation of techniques for assessment of mammal populations in Wisconsin. *In* Scott Jr NJ, editor. Mammal communities. 2nd ed. Pergamon (New York). p 122-30.

Dissertations and Theses – General format is given first.

Author. Date of degree. Title [type of publication – dissertation or thesis]. Place of institution: name of institution granting the degree. Total number of pages. Availability statement.

The availability statement includes information about where the document can be found or borrowed if the source is not the institution's own library.

Stevens WB. 2004. An ecotoxilogical analysis of stream water in Arkansas [dissertation]. State University (AR): Arkansas State University. 159 p.

Millett PC. 2003. Computer modeling of the tornado-structure interaction: Investigation of structural loading on a cubic building [MS thesis]. Fayetteville (AR): University of Arkansas. 176 p. Available from: University of Arkansas Microfilms, Little Rock, AR; AAD74-23.

Stevens WB. 2004. An ecotoxilogical analysis of stream water in Arkansas [dissertation]. State University (AR): Arkansas State University. 159 p.

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Vivian VL, editor. 1995. Symposium on Nonhuman Primate Models for AIDS; 1994 June 10-15; San Diego, CA. Sacramento (CA): Grune & Stratton. 216 p.

Scientific and Technical Reports – General format is given first.

Author(s) (Performing organization). Date of publication. Title. Type report and dates of work. Place of publication: publisher or sponsoring organization. Report number. Contract number. Total number of pages. Availability statement if different from publisher or sponsoring organization. (Availability statement may be an internet address for government documents.)

Harris JL and ME Gordon (Department of Biological Sciences, University of Mississippi, Oxford MS). 1988. Status survey of *Lampsilis powelli* (Lea, 1852). Final report 1 Aug 86 – 31 Dec 87. Jackson (MS): US Fish and Wildlife Service, Office of Endangered Species. Report nr USFW-OES-88-0228. Contract nr USFW-86-0228. 44+ p.

Electronic Journal Articles and Electronic Books should be cited as standard journal articles and books except add an availability statement and date of accession following the page(s):

653 p. Available at: www.usfw.gov/ozarkstreams. Accessed 2004 Nov 29.

Online resources

Citation depends on the requirement of the particular website. Otherwise use the “electronic journal article” format.

US Geological Survey (USGS). 1979. Drainage areas of streams in Arkansas in the Ouachita River Basin. Open file report. Little Rock (AR): USGS. 87 p. <www.usgs.gov/ouachita> Accessed on 2 Dec 2005.

Cited in text as: (USGS 1979)

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(Harris and Gordon 1988, Steiner et al. 1992, Johnson 2006).

8. Submission of Obituaries and *In Memoria*

The Executive Committee and the Journal of the Arkansas Academy of Science welcome the opportunity to pay appropriate professional honor to our departed Academy colleagues who have a significant history of service and support for the Academy and Journal. The editorial staff will consider obituaries for former executive committee members to be included in the Journal. Additional obituaries not meeting these criteria will be forwarded to be posted on the Academy website. We would request that paid up members of the Academy that wish to write an obituary provide a one to two page professional description of the scientist's life that should include details of his/her contribution to the Academy and publication record. The format should follow the two column format and 11pt Times New Roman font. A color or black-and-white photograph to fit in one column should also be provided.

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Evaluation of a paper submitted to the *JOURNAL* begins with critical reading by the Managing Editor. The manuscript is then submitted to referees for critical review for scientific content, originality and clarity of presentation. To expedite review, authors should provide the names and current e-mail address of at least three reviewers within the appropriate field, with whom they have not had a collaboration in the past two years. Potential reviewers that the authors wish to avoid due to other conflicts of interest can also be provided. Attention to the preceding paragraphs will also facilitate the review process. Reviews will be returned to the author together with a judgement regarding the acceptability of the manuscript for publication in the *JOURNAL*. The authors will be requested to revise the manuscript where necessary. Time limits for submission of the manuscript and publication charges will be finalized in the accompanying letter from the Managing Editor (see “Proposed timetable for manuscript processing”). The authors will then be asked to return the revised manuscript, together with a cover letter detailing their

Instructions to Authors

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